AN ABSTRACT OF THE THESIS OF

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Title: RELATION OF THE DECOMPOSITION OF TRIMETHYLAMINE OXIDE AND THE QUALITY OF PACIFIC SHRIMP (PANDULUS JORDANI)

Abstract approved: David L. Crawford

The relationship between the decomposition of trimethylamine oxide in Pacific shrimp and shrimp meat quality was investigated to evaluate the use of the trimethylamine oxide system as a quality control indice.

Changes in the levels of trimethylamine oxide and its decomposition products in whole shrimp stored on ice and in its derived raw and cooked meat and in raw shrimp meat stored under refrigeration (1-2° C) were investigated. The concentration dependency of the decomposition of trimethylamine oxide to dimethylamine and formaldehyde in frozen storage (-18° C) and the heat sensitivity of the trimethylamine oxide decomposition system was evaluated. A statistical relationship between amine and formaldehyde levels in whole shrimp and raw and cooked meat stored under laboratory conditions and obtained from commercial processing plants with flavor panel

scores was developed.

Trimethylamine oxide levels decreased in a linear manner in whole shrimp, and in the raw and cooked meat during iced storage. This apparent disappearance was related to the washing action of melting ice and its degradation to trimethylamine, dimethylamine and formaldehyde. Levels of dimethylamine and formaldehyde increased in a parallel manner during iced storage. Trimethylamine levels increased steadily during the first four days of storage, followed by rapid increase during the latter four days reflecting a microbial out-growth.

Trimethylamine oxide levels in raw shrimp meat held at 1-2° C decreased during the first four days of storage at a relatively slow rate, followed by a sharp decline during the remainder of an eight day storage period. Trimethylamine levels were shown to remain relatively constant during the first four days of storage, followed by a rapid increase in levels reflecting the decline of trimethylamine oxide levels. Dimethylamine and formaldehyde levels increased rapidly in a linear manner during the storage period.

Dimethylamine and formaldehyde levels in frozen raw shrimp meat increased during storage (-18° C) in a linear manner. The rate of dimethylamine formation was shown to depend upon initial trimethylamine oxide levels and/or enzyme concentration. A rate dependency on trimethylamine oxide for formaldehyde was not

established. The presumably enzyme-catalyzed mechanism of dimethylamine formation was found to be completely inactivated by the exposure of the raw meat to water at 100° C for 15 seconds.

Flavor panel scores for cooked meat derived from whole shrimp stored on ice declined in a linear manner over an eight day storage period. Scores for these samples of shrimp meat and samples obtained from commercial processing plants correlated well with trimethylamine oxide, dimethylamine, and formaldehyde levels in whole shrimp and derived raw and cooked meat. Trimethylamine levels, indicative of microbial out-growth provided correlations inferior to these indices.

The magnitude of change observed in trimethylamine oxide levels accurately reflected flavor panel scores. The simplicity of its determination supports its use in quality control practices.

Relation of the Decomposition of Trimethylamine Oxide and the Quality of Pacific Shrimp (*Pandalus jordani*)

by

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INTRODUCTION

Pacific shrimp is an important seafood resource of the west coast states. Landings in Oregon in 1972 amounted to over 20.7 million pounds with a value of approximately three million dollars (Fishery Statistics of the United States, 1972).

Objective quality tests are urgently needed by the shrimp industry and the regulatory agencies to insure the marketing of products of consistently good quality. Such tests can help the industry to process and distribute shrimp products according to their quality and their expected storage life.

Generally, organoleptic measurements of quality have been used to grade shrimp products at different stages during their storage life. Since organoleptic evaluations are subjective and many times dependent upon the qualifications, the health and emotional state of judges as well as environmental factors, it is difficult to compare tests. These differences make it desirable to supplement organoleptic testing with an objective test for quality.

Numerous studies have been made correlating biochemical changes postmortem in shrimp to loss of quality, but not a single satisfactory test has been developed for determining the relative

quality of shrimp through all the stages from prime freshness to definitive spoilage. Only a few tests have been found useful for indicating the quality at some particular stage of iced storage.

A recent study (Flores and Crawford, 1973) of the quality changes in iced Pacific shrimp (Pandalus jordani) indicated that the formation of dimethylamine and formaldehyde might reflect the changes in the quality of cooked meat. Because of the magnitude of change in these compounds with iced storage, the authors suggested that its determination could be a sensitive test of the organoleptic quality of the shrimp meat.

The objectives of this research were:

1. Investigate the kinetics of the decomposition of trimethylamine oxide to dimethylamine and formaldehyde in raw and cooked Pacific shrimp meat during frozen storage.
2. Investigate the decomposition of trimethylamine oxide in whole raw shrimp during iced storage and in raw refrigerated meat.
3. Correlate flavor panel scores on shrimp meat with its levels of trimethylamine oxide and its products of decomposition.

LITERATURE REVIEW

Deterioration of shrimp quality is generally considered to result from the combined action of enzymes from either the tissues or contaminating microorganisms, chemical reactions and physical handling (Fieger and Friloux, 1954; Fieger et al., 1958; Flick and Lovell, 1972). Numerous studies have been made of the biochemical changes that occur in shrimp after death to develop a chemical basis for objective tests which would correlate numerically with the shrimp meat quality. None of these tests after thorough investigation has proven in itself completely satisfactory for evaluating the deterioration of quality from prime to spoiled.

Organoleptic studies made by Fieger and Friloux (1954) with shrimp stored in ice showed that definite flavor changes occur in three phases. During the first days of ice storage the sweet flavor characteristic of fresh shrimp changes only slightly; this phase is followed by a period during which the shrimp has a flat taste or are tasteless, but no off flavors associated with spoilage are noted. The second phase is followed by another sharp change in flavor. At this time, off flavors characteristic of spoilage are evident.

It has been established that the loss of quality during the early periods of storage is mainly caused by autolysis, and with longer storage, spoilage occurs mainly through bacterial action (Fieger and

Frilous, 1954). However, certain palatability attributes such as texture and juiciness have been observed to improve during the three first days of ice storage probably due to the autolytic changes (Flores and Crawford, 1973).

Some of the tests that have been advocated as indices of shrimp quality include pH, amino nitrogen, trimethylamine, total volatile bases, volatile acids, aerobic plate count, degree of hydration of water soluble proteins, picric acid turbidity test, catechol-ferric chloride reaction, carotenoid content, indole, glycogen-sugar, lactic acid, soluble orthophosphate, B-vitamin content, free fatty acids, peroxide number, and more recently hypoxanthine, dimethylamine, formaldehyde and the total volatile bases/amino nitrogen ratio.

Of all the tests indicated, only pH is used to some extent by the shrimp industry in the United States. In general it is not considered as a reliable index and its interpretation is confusing. Bailey et al. (1956) classified iced shrimp as having prime quality with a pH of 7.7 or below; poor quality, but acceptable with values from 7.7 to 7.95 and spoiled with pH levels of 7.95 or above. Iyengar et al. (1960 reported that a pH value of 7.1 or less inside the muscle of shrimp indicated prime quality; values from 7.2 to 7.6 indicated a spoilage stage, and values greater than 7.6 definite spoilage. Bethea and Ambrose (1962) found that shrimp with pH from 7.24 to 7.8 were of good quality and shrimp with pH levels of 8.0 to 8.2 possessed fair to borderline quality with spoilage

indicated by a pH above 8.2. Flores and Crawford (1973) reported a progressive increase in pH from 7.6 to 8.8 for intact Pacific shrimp during eight days of iced storage, but they also found the initial pH to vary enormously within lots of shrimp.

The increase in bacterial counts is considered useful for determining the onset of spoilage, but for quality control work is considered to require too much time. Green (1949) reported bacteriological data on shrimp from the time they were caught until marketed as fresh or frozen. Campbell and Williams (1952) made quantitative and qualitative observations on both the internal as well as the external flora of headless iced shrimp; they indicated that satisfactory quality can be retained for at least 16 days. During the storage period, they found an increase of psychrophilic bacteria with Achromobacter being the dominant genus at the end of the storage. Fieger and Friloux (1954) and Iyengar et al. (1960) found good correlation between total plate counts and organoleptic scores. The increase in total plate counts was found to precede similar relative increases in volatile acids, total volatile bases and trimethylamine levels. Flores and Crawford (1973) reported an increase in the bacterial load of shrimp during eight days of ice storage, but the total plate counts were found to fall within the range from 3.0×10^5 and 1.3×10^6 organisms per g found in commercial samples of fresh Pacific shrimp by Harrison and Lee (1969).

Amino nitrogen has been reported to have considerable merit as an index of quality for shrimp, but the reports are contradictory. Campbell and Williams (1952); Flores and Crawford (1973); Cobb et al. (1973); and Cobb and Vanderzant (1975) reported a decrease of amino nitrogen with storage time. Fieger and Friloux (1954) found an increase of amino nitrogen with time of storage and reported that this correlated well with flavor scores.

Total volatile bases and trimethylamine have been also the subject of contradictory reports. Iyengar et al. (1960) found these tests unreliable and of limited usefulness as indices of quality for ice stored shrimp. Fieger and Friloux (1954) found that trimethylamine was only of value to indicate whether or not spoilage had occurred. Bethea and Ambrose (1962), reported that the trimethylamine levels in iced shrimp were of little value for the determination of changes of prime quality. Flores and Crawford (1973) found an increase of trimethylamine levels with storage but they concluded that due to the magnitude of change the levels of trimethylamine could not be used as a precise index of quality. Collins et al. (1960) found that trimethylamine and total volatile bases levels together with volatile acids correlated very well with subjective ratings of shrimp stored in ice and refrigerated sea water. Ganon and Fellers (1958) suggested that total volatile bases and the ratio of total volatile bases to total nitrogen were good indicators of quality for frozen

breaded shrimp. Cobb and Vanderzant (1975) reported that the total volatile bases/amino nitrogen ratio correlate well with the quality of shrimp, they also reported that in some cases the individual values of amino nitrogen and total volatile bases can be more valuable in assessing the shrimp quality than the ratio per se. They suggested some guidelines for using this test which included that samples with a total volatile base content higher than 30 mg/100 g be regarded as spoiled regardless of the ratio of total volatile bases to amino nitrogen. Total volatile bases and trimethylamine are used in Germany, in some sectors of the Australian marketing system and Japan, which has established as limit of acceptability five mg of trimethylamine and/or 30 mg of total volatile bases.

The picric acid turbidity test was reported by Bethea and Ambrose (1962), and Ambrose et al. (1964) as a fairly good method to determine the freshness of the shrimp but did not correlate very well with organoleptic evaluations. They found that the increase in readings for the picric acid turbidity test coincided with an increase of trimethylamine levels.

The carotenoid content has been proposed by Collins and Kelley (1969) and Kelley and Harmon (1972) as an index of shrimp quality. Flores and Crawford (1973) found that the carotenoid content did not correlate well with the storage time.

Changes in total nitrogen and non protein nitrogen have been

used as indices for determining the freshness of shrimp (Fieger and Friloux, 1954; Ganon and Fellers, 1958; Collins et al., 1960; Flores and Crawford, 1973). The results of these investigations showed variable relationships between these indices and the degree of freshness of iced shrimp. Free tyrosine was found by Fieger and Friloux (1954) to vary widely and irregularly. Flores and Crawford (1973) found that the tyrosine levels in the cooked meat increased during the storage period reflecting the proteolytic activity in the shrimp meat.

Iyengar et al. (1960) reported that the catechol-ferric chloride test is a good index of the quality of ice stored shrimp.

Many other tests have been found useful to determine the quality of shrimp during some phase of its deterioration. Bailey et al. (1954) indicated that glycogen sugar content, lactic acid, and acid soluble orthophosphate could be used for "relative" comparison during the period of prime quality. They suggested that for other phases of the deterioration of shrimp the B-vitamin content, free sulfhydryl groups, and the degree of hydration of water soluble proteins were good indices.

Shelef and Jay (1971) reported that the degree of hydration of the shrimp proteins determined as the extract release volume was a good index of the microbiological quality. Vanderzant and Nickelson (1971) did not find good correlation between the extract release volume

and the microbiological quality of shrimp.

Flick and Lovell (1972) suggested that the hypoxanthine levels may be a good indicator of the quality of shrimp; they speculated that the flavor deterioration could be related to the production of inosine and hypoxanthine, plus the concurrent loss of inosine monophosphate. Unpublished results of work done with Pacific shrimp (Pandalus jordani) in the Seafood Laboratory of Oregon State University, have shown that the loss of inosine monophosphate correlates very well with the quality of shrimp (Babbitt, 1975).

During the last five years, many investigations of the degradation of trimethylamine oxide to dimethylamine and formaldehyde during frozen storage of fish of the family Gadidae have been carried out. Tokunaga (1964) observed this phenomenon in pollock during frozen storage. Castell et al. (1970) have extended this observation to cod, haddock, and cusk. Babbitt et al. (1970) found an increase of dimethylamine and formaldehyde during the frozen storage of hake. Amano and Yamada (1974) found increases of dimethylamine and formaldehyde in Gadoid fish stored at refrigeration temperatures.

Japanese workers have isolated an enzyme system from fish and shellfish that reduces trimethylamine oxide to dimethylamine and formaldehyde and have studied some of its characteristics (Yamada and Amano, 1965a, 1965b; Yamada et al., 1969; Harada and Yamada, 1971).

Very little work has been done in this respect with shrimp. Sundsvold et al. (1969a and 1969b) investigated the trimethylamine oxide content and its decomposition in canned Norwegian shrimp. High levels of the oxide in raw and fresh cooked shrimp were shown to be reduced after canning and storage. Trimethylamine and dimethylamine contents were shown to increase and were accompanied by the formation of formaldehyde. Values of pH 5.5 or lower and/or the addition of reduced iron, copper sulfate and titanous chloride to the canned product accelerated the reduction of the trimethylamine oxide with the subsequent formation of formaldehyde and di- and trimethylamine during storage. The quantitative relationship between formaldehyde and dimethylamine formed did not support a straightforward fission of the oxide to this compound. Flores and Crawford (1973) found a steady increase of dimethylamine and formaldehyde, and reduction of trimethylamine oxide during the iced storage of shrimp and suggested that the determination of dimethylamine and formaldehyde can be used as a sensitive test that could better reflect the organoleptic quality of the shrimp meat. Castell et al. (1970) reported that in shrimp meat there was no reduction of trimethylamine oxide to dimethylamine and formaldehyde during frozen storage.

EXPERIMENTAL

Materials and Handling Procedures

All samples of shrimp used in this investigation were obtained from commercial plants in Astoria, Oregon during the summer of 1975. Because of the experimental design, a series of samples were obtained and handled in different ways.

A 60 pound lot of one day old Pacific shrimp used to investigate the kinetics of decomposition of trimethylamine oxide in raw shrimp meat during frozen and refrigerated storage was obtained on July 22, 1975. To ascertain the effects of the initial levels of trimethylamine oxide during frozen storage, the lot of whole shrimp was divided into three equal portions. Two of the portions of whole shrimp were well iced and stored under refrigeration at 1-2° C. The remainder of the whole shrimp was sampled in triplicate for chemical analysis and peeled by hand. The raw meat was washed, sampled in triplicate for chemical analysis, divided into small portions of approximately 40 g, put into small polyethylene bags and closed with rubber bands. One group of the packaged shrimp samples was frozen overnight at -27° C, then stored at -18° C. The second group of packaged raw shrimp meat was stored at 1-2° C and analyzed daily for a period of eight days. Four days later one of the portions of the whole shrimp stored on ice was processed as previously described, with the

exception that raw shrimp meat was not stored at 1-2° C. After eight days of iced storage the third portion of the whole shrimp was processed in a manner similar to the second sample. Each lot of frozen raw meat was analyzed in triplicate at spaced intervals for 105 days.

On July 23 of 1975 a sample of whole shrimp less than one day old was obtained. This shrimp was used to investigate the effect of the cooking time in the decomposition of the trimethylamine oxide during frozen storage. The whole shrimp was peeled by hand, and the separated raw meat was washed and divided into two equal portions which were cooked in water at 100° C for 15 and 30 seconds, respectively. The cooked shrimp meat was sampled in triplicate for chemical analysis while the remainder was divided in portions of approximately 30 g, placed into small polyethylene bags and closed with rubber bands. These samples were then frozen and stored in the manner previously described for the raw shrimp meat. The frozen meat was analyzed in triplicate at spaced intervals for 45 days.

On September 25, 1975, one lot of 120 pounds of whole shrimp less than one day old was obtained and divided in six 20 pound portions. Five portions of the shrimp were well iced and stored at 1-2° C. The remaining portion of whole shrimp was sampled from the surface, the middle and the bottom of the container in which it

was transported to the laboratory. The whole shrimp was well mixed and a part of it was hand peeled to yield a sample of raw meat. Whole shrimp and raw meat were sampled (40 g) in triplicate for chemical analysis. The remainder of the whole shrimp was cooked for two minutes at 100° C, the meat picked from the shell by hand, washed and put into styrofoam containers. The cooked meat was then forzen overnight at -27° C, the containers vacuum sealed in moisture vapor proof film and stored at -18° C until they were transported to the Department of Food Science and Technology in Corvallis, Oregon where they were subjected to flavor panel evaluations. Prior to flavor panel evaluations triplicate samples were chemically analyzed. Each portion of the whole shrimp stored on ice was processed as previously described after two, four, six, and eight days of storage.

At various times throughout the processing season a total of seven samples of shrimp were collected from two commercial processing plants. In each case, samples of whole raw shrimp representative of the lots before processing and enough cooked, machine peeled shrimp meat derived from the same lots of whole shrimp for flavor panel evaluations and chemical analysis were obtained. The samples were rapidly transported to the laboratory where the cooked meat was cleaned and processed as previously described for the cooked meat from the whole shrimp stored on ice.

Whole shrimp were sampled immediately and cooked meat just prior to flavor panel evaluations in triplicate for chemical analyses.

Extraction Procedure

In all cases, the samples of shrimp were blended for two minutes in a Waring Blender with enough 6.25% trichloroacetic acid to have a final dilution 1:5 (final TCA concentration of 5%). The trichloroacetic acid homogenates were allowed to stand for 30 minutes at 4° C and then filtered through a S & S #588 filter paper and stored at -18° C prior to analysis.

Analysis for Trimethylamine, Trimethylamine Oxide and Formaldehyde

Trimethylamine was determined using the picric acid procedure of Dyer (1945) as modified by Murray and Gibson (1972) with the exception that the tubes were shaken by hand 150 times instead of 40 times. In preparatory work, it was found that shaking the tubes the recommended 40 times did not give good replications. The results were calculated with a regression equation calculated with solutions of trimethylamine hydrochloride previously standardized by a semi-micro Kjeldahl procedure (A. O. A. C. 1970).

Trimethylamine oxide was determined using a modification of the procedure of Yamagata et al. (1969) involving the reduction

of trimethylamine oxide to trimethylamine. One ml of the trichloroacetic acid extracts was diluted to 10 or 20 ml with distilled water, depending on the levels of trimethylamine oxide and trimethylamine expected. One ml of this dilution was transferred to a screw-top culture tube with a teflon cap and 0.5 ml of 1% titanium chloride was added. The tube was capped, put into a water bath at 80±1° C for five minutes to accomplish the reduction. The reaction mixture was cooled with cold water, 2.5 ml of distilled water was added and subjected to trimethylamine analysis. The results in this determination represented total trimethylamine (trimethylamine oxide + trimethylamine). The trimethylamine oxide was calculated by subtracting the trimethylamine value from the total trimethylamine and multiplying the result by 1.270681779 to express the results as the oxide.

Formaldehyde was determined in the trichloroacetic acid extracts using the method of Sawicki et al. (1961) as modified by Babbitt et al. (1972). Standard solutions of formaldehyde were prepared from analytical reagent grade formaldehyde solution which was standardized using the sodium sulfite method described by Walker (1964).

All the analyses were carried out in triplicate.

Analysis of Dimethylamine

Dimethylamine was determined using the method of Dyer and

Mounsey (1945) on distillates of trichloroacetic acid extracts. The direct application of this method to trichloroacetic acid extracts was not possible because a yellow interference developed after adding the reactants which interfered with the extraction of the dimethyldithiocarbamate copper salt making impossible the quantitative estimation of dimethylamine.

To avoid this interference, the amines were steam distilled into an acid solution from which dimethylamine was determined. An aliquot of the trichloroacetic acid extracts (usually five ml) was placed in a micro-Kjeldahl unit with five ml of 45% sodium hydroxide and a few drops of antifoam. The amines were distilled and 20 ml of condensate were collected in a graduate cylinder containing five ml of 0.15 N hydrochloric acid. Care was taken to assure that the tip of the condenser was under the acid solution before adding the base. The distillates were thoroughly mixed and dimethylamine was determined using a 10 ml aliquot. The concentration of dimethylamine was calculated using regression equation developed by applying the method to solutions of dimethylamine hydrochloride standardized by a semi-micro Kjeldahl procedure (A.O.A.C. 1970).

To verify the quantitative distillation of dimethylamine in the range of concentrations expected in trichloroacetic acid extracts, recovery experiments were carried out. Aliquots of tichloroacetic acid extracts were analyzed before and after adding known amounts

of dimethylamine. Each recovery experiment was carried out in duplicate and the results were analyzed statistically using methods outlined by Kramer and Twigg (1970).

The results of the recovery experiments (Table 1) showed that dimethylamine was quantitatively distilled. The average recovery was 98.21% with a coefficient of variability for the method of 3.25%. Standard curves developed from distilled standard solutions and from standard solutions directly were almost identical (Figure 1).

Table 1. Recovery of added dimethylamine from trichloroacetic extracts of whole Pacific shrimp by steam distillation.

Sample	Dimethylamine · HCl (μg) Added	Found	Recovery %
A	-	8.839	-
A	5.052	13.349	96.10
A	5.052	13.869	99.84
A	16.840	26.271	102.30
A	16.840	24.392	94.99
B	-	26.646	-
B	25.260	54.832	105.64
B	25.260	49.195	94.78
B	33.680	60.845	100.86
B	33.680	57.463	95.40
C	-	23.264	-
C	5.052	27.400	96.77
C	5.052	27.022	95.43
Average			98.21

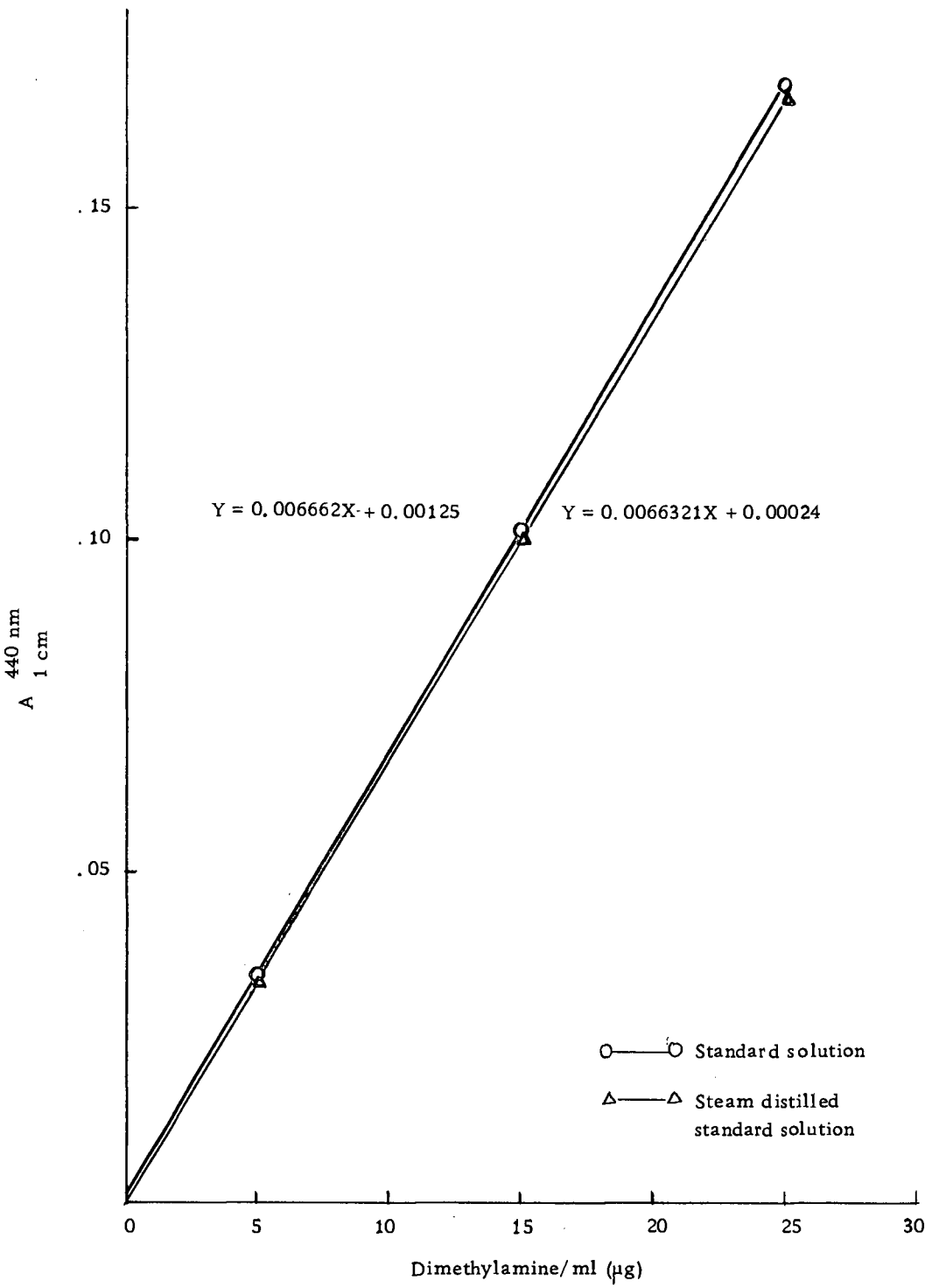

Figure 1. Absorbancy of dimethylthiocarbamate copper salts formed by dimethylamine standard solutions and by steam distillates of standard solutions.

Flavor Panel Evaluations

The frozen samples of cooked shrimp meat for flavor panel evaluations were transported in insulated containers to the Department of Food Science and Technology in Corvallis, Oregon. Samples were thawed overnight at 1-2° C then evaluated by a flavor panel, each composed of 15 staff members of the Department, in triplicate. Panelists were asked to judge the shrimp meat for texture, juiciness, degree of off-flavor and overall desirability using a 9-point hedonic scale, ranging from 9, "extremely desirable," to 1, "extremely undesirable." The scores for the triplicate flavor panels were combined by organoleptic factor and treatment and evaluated by analysis of variance. The significance of individual treatment means was determined by Duncan's multiple range tests. Mean chemical values were correlated with mean scores for each of the triplicate flavor panel evaluations of corresponding samples using linear regression procedures.

RESULTS AND DISCUSSION

Decomposition of Trimethylamine Oxide in Iced Whole Shrimp

The occurrence and distribution of trimethylamine oxide in marine fish has been investigated since 1900 because of its bacterial reduction to trimethylamine. Trimethylamine oxide has been found in most kinds of fin and shellfish (Beatty, 1939; Norris and Benoit, 1945; Dyer, 1951), in the ordinary and bloody muscle of tuna (Yamagata et al., 1969), and in several other species of fish (Tokunaga, 1970).

The trimethylamine oxide content of Pacific shrimp was investigated by Flores and Crawford (1973). Trimethylamine has been proposed as an index of seafoods quality and the accumulation of this compound during iced storage of shrimp has been investigated by Fieger and Friloux (1954), Bailey et al. (1956), Iyengar et al. (1960), Campbell (1962) and Flores and Crawford (1973). Formation of trimethylamine has been linked to the action of bacterial enzymes which reduce trimethylamine oxide to trimethylamine (Tarr, 1939, 1940) and a close relationship between the numbers of bacteria in shrimp muscle and the amount of trimethylamine formed has been reported by Fieger and Friloux (1954), Bailey et al. (1956) and Iyengar et al. (1960). Amano and Yamada (1964) suggested from

their investigation of gadoid fish an enzymatic system for the reductive degradation of trimethylamine oxide to dimethylamine and formaldehyde.

Figures 2 through 5 show the regression lines which express the trimethylamine oxide, trimethylamine, dimethylamine and formaldehyde levels, respectively, in whole shrimp during iced storage. Figures 6 through 9 and 10 through 13 show corresponding levels for the raw and cooked meat, respectively, derived from whole shrimp.

Trimethylamine oxide levels in whole shrimp (Figure 2), raw meat (Figure 6) and cooked meat (Figure 10) decreased in a linear manner ($P < 0.005$) during the storage period.

Levels of trimethylamine, dimethylamine and formaldehyde in whole shrimp (Figures 3, 4 and 5, respectively), raw meat (Figures 7, 8 and 9, respectively) and cooked meat (Figures 11, 12 and 13, respectively) increased linearly ($P < .005$) with storage time.

The decrease in trimethylamine oxide levels reflects its degradation to trimethylamine, dimethylamine and formaldehyde and a large loss due to the washing action of melting ice. These results are in agreement with the findings of Flores and Crawford (1973) who reported large losses of trimethylamine oxide which could not be accounted for by its degradation.

Trimethylamine oxide levels in whole shrimp and cooked meat were very similar, but considerably lower than in the raw meat

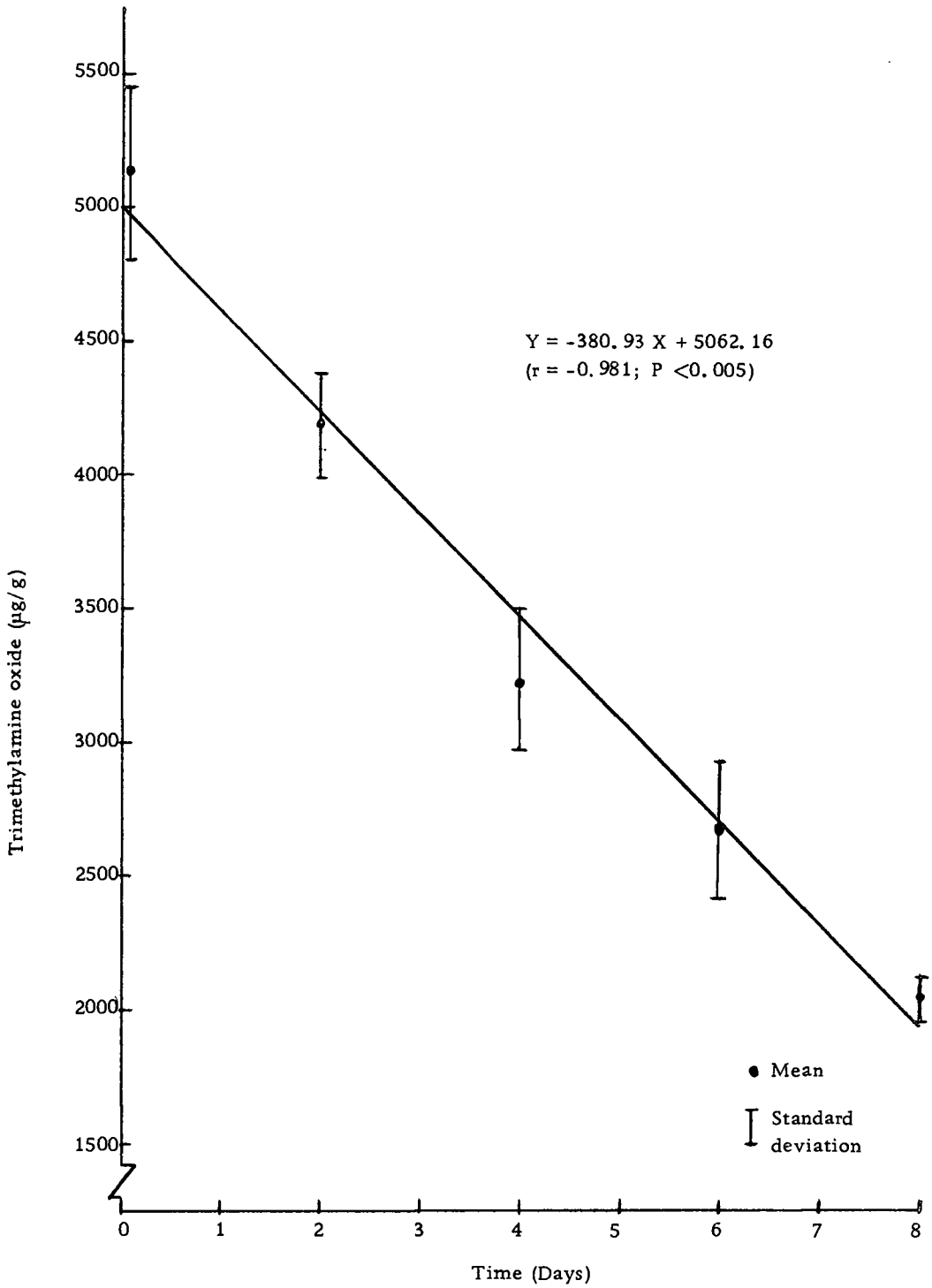

Figure 2. Regression of trimethylamine oxide content of iced whole shrimp on storage time.

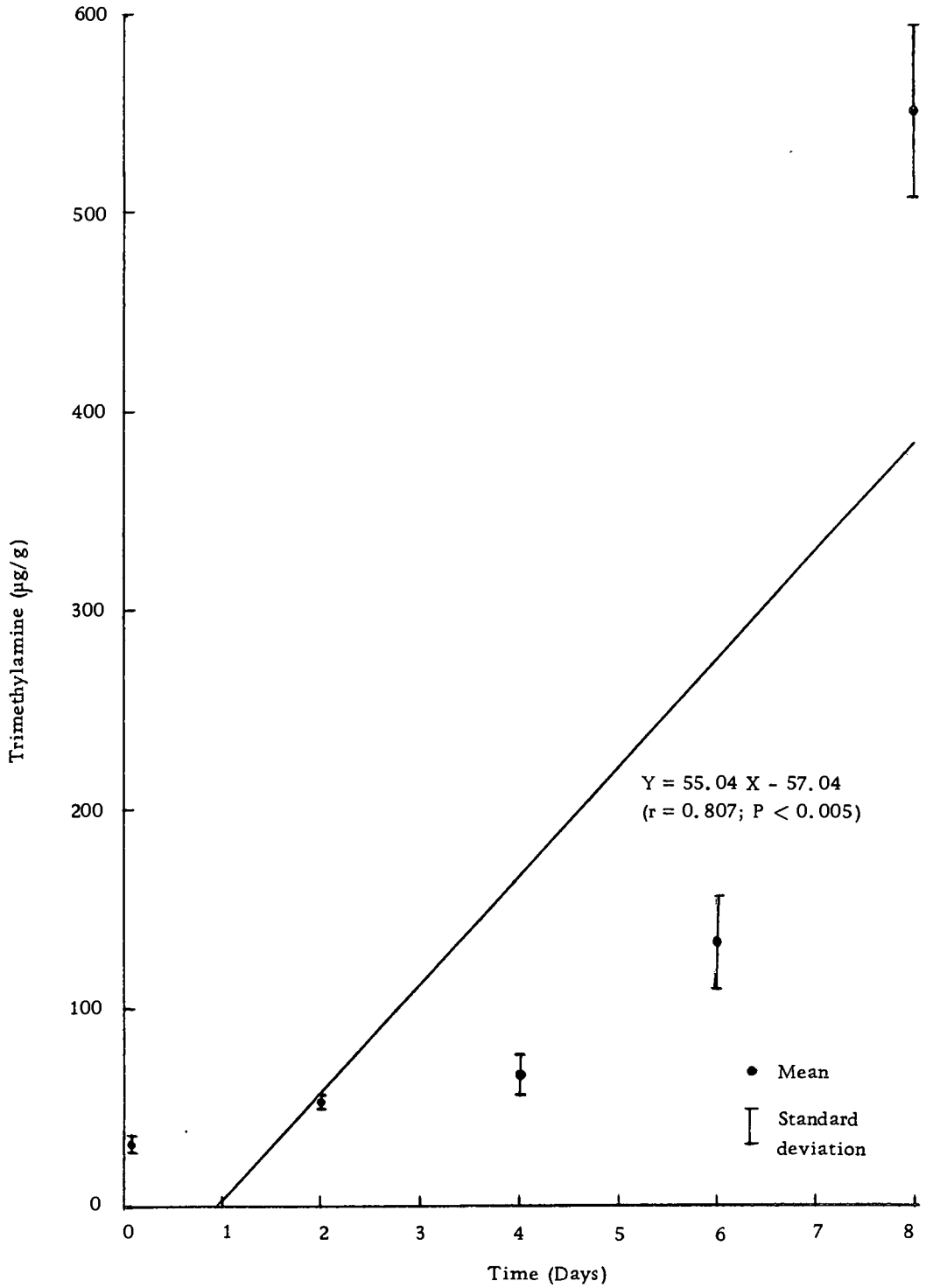

Figure 3. Regression of trimethylamine content of iced whole shrimp on storage time.

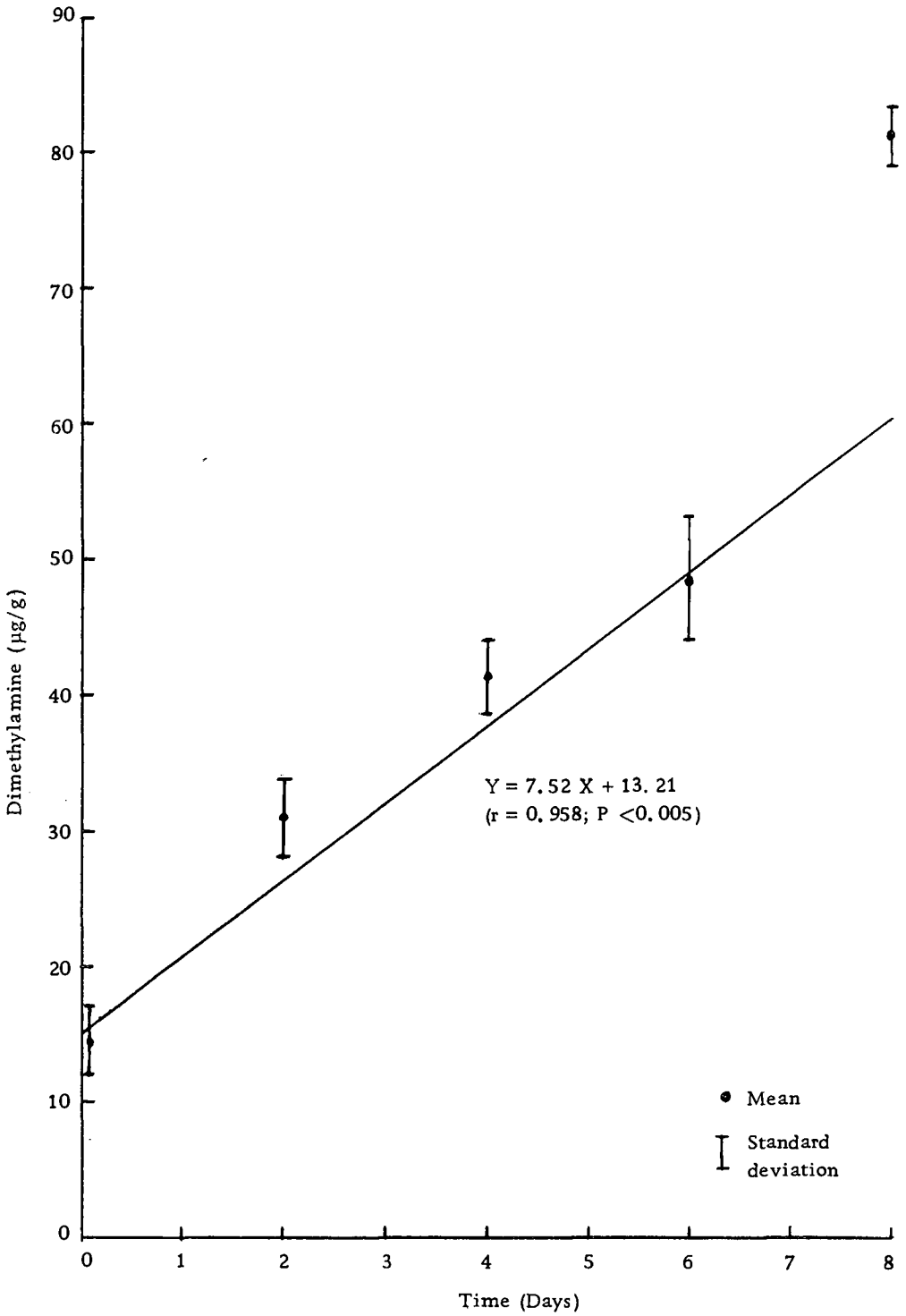

Figure 4. Regression of dimethylamine content of iced whole shrimp on storage time.

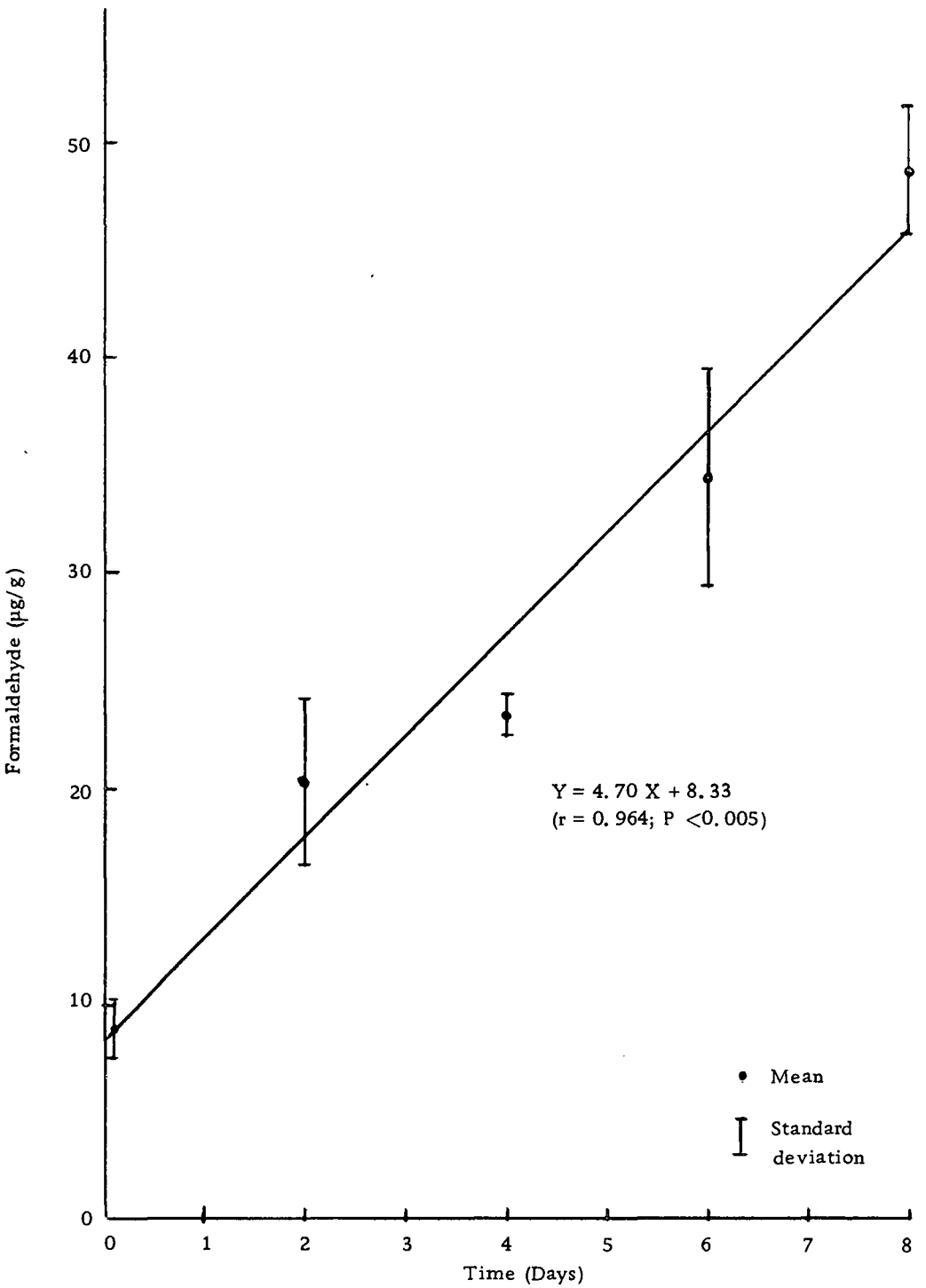

Figure 5. Regression of formaldehyde content of iced whole shrimp on storage time.

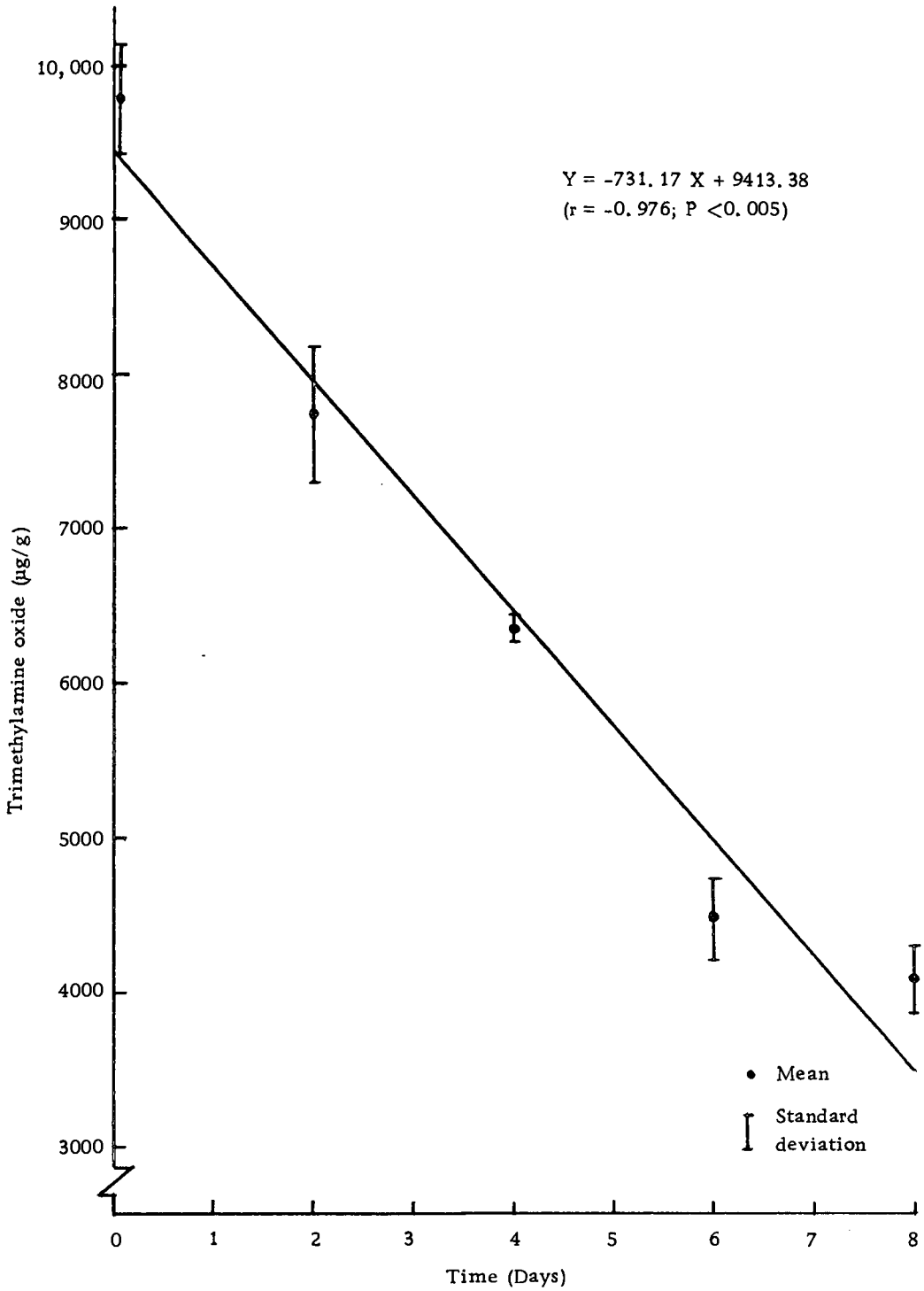

Figure 6. Regression of trimethylamine oxide of raw meat derived from iced whole shrimp on storage time.

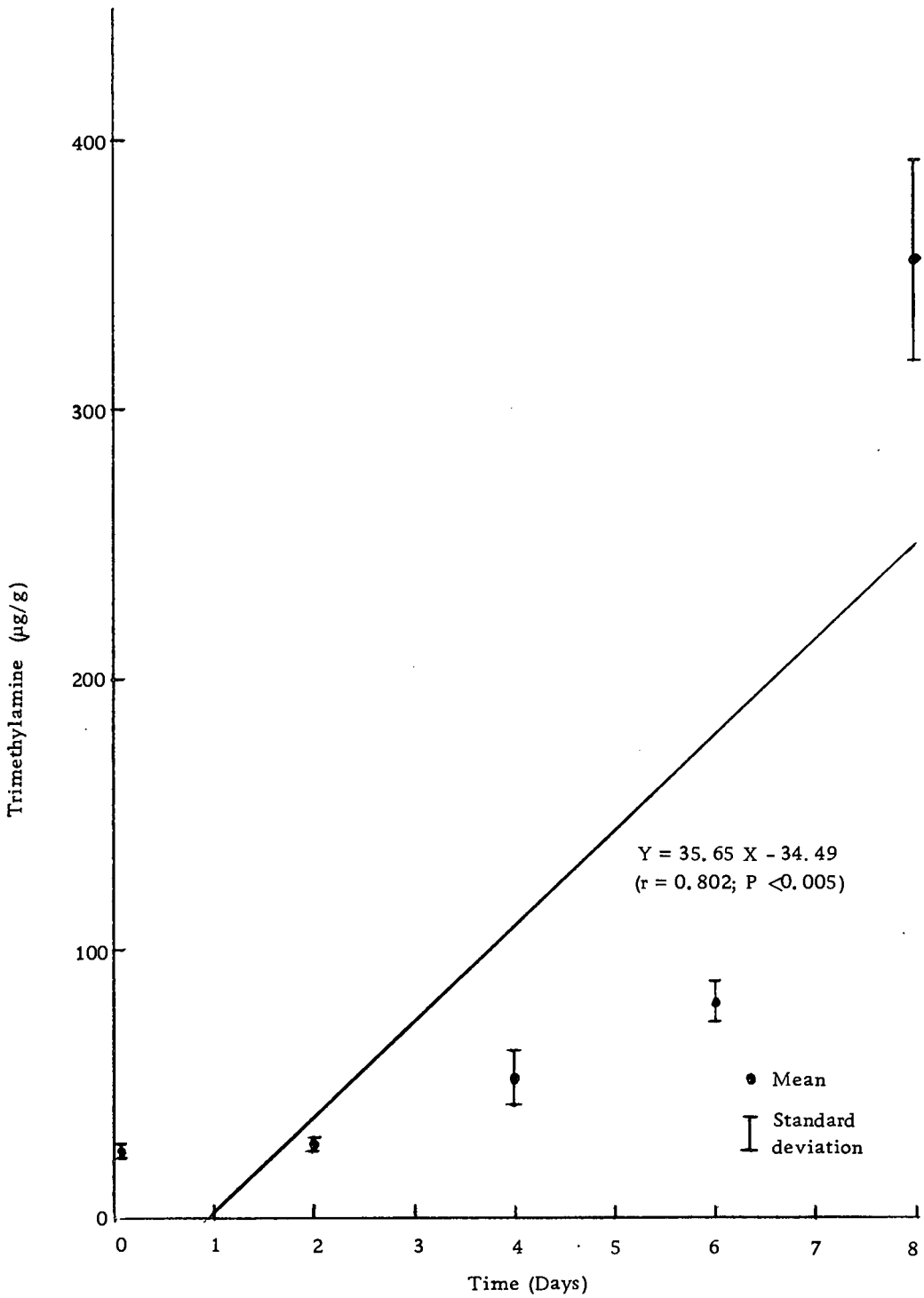

Figure 7. Regression of trimethylamine of raw meat derived from iced whole shrimp on storage time.

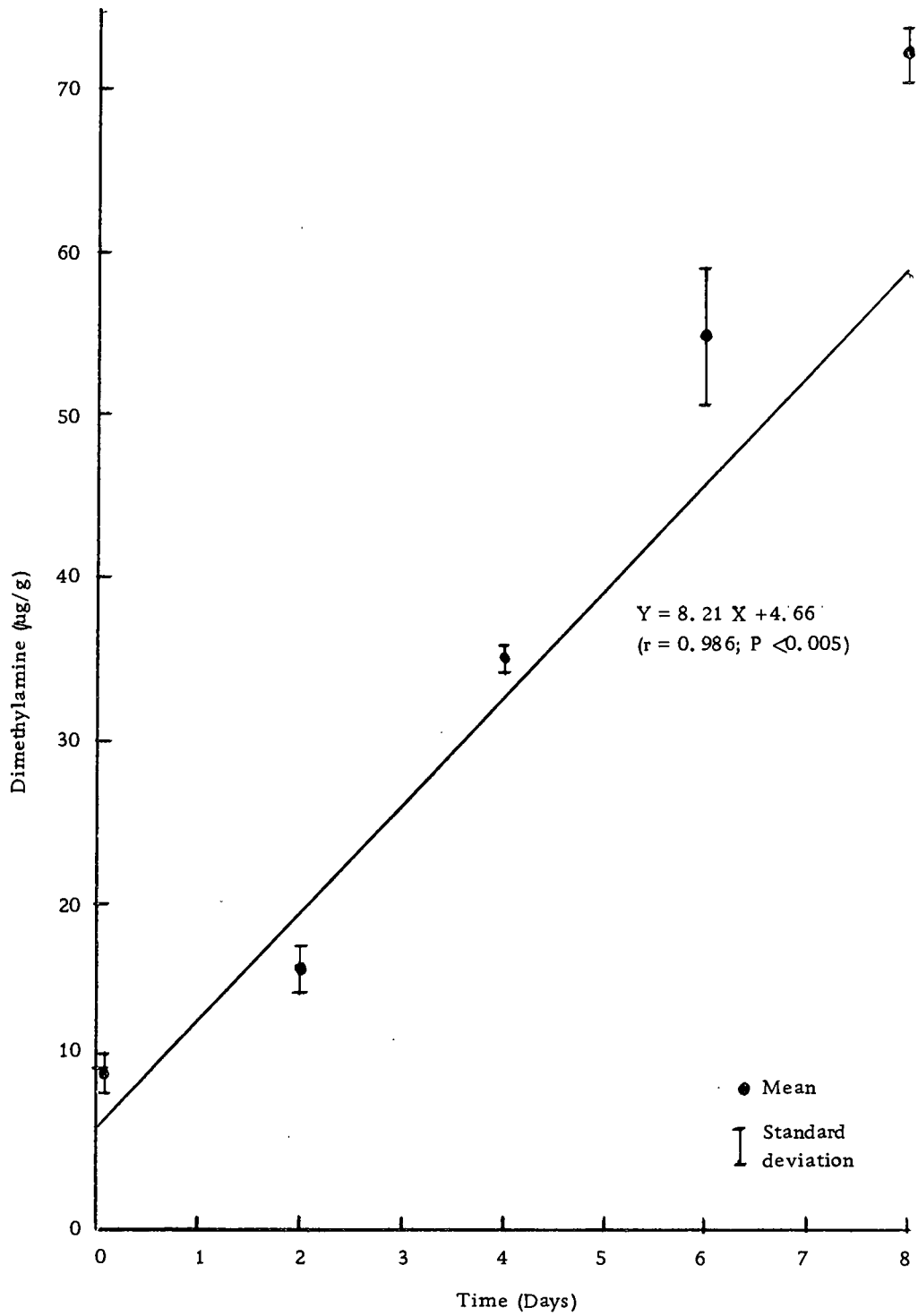

Figure 8. Regression of dimethylamine of raw meat derived from iced whole shrimp on storage time.

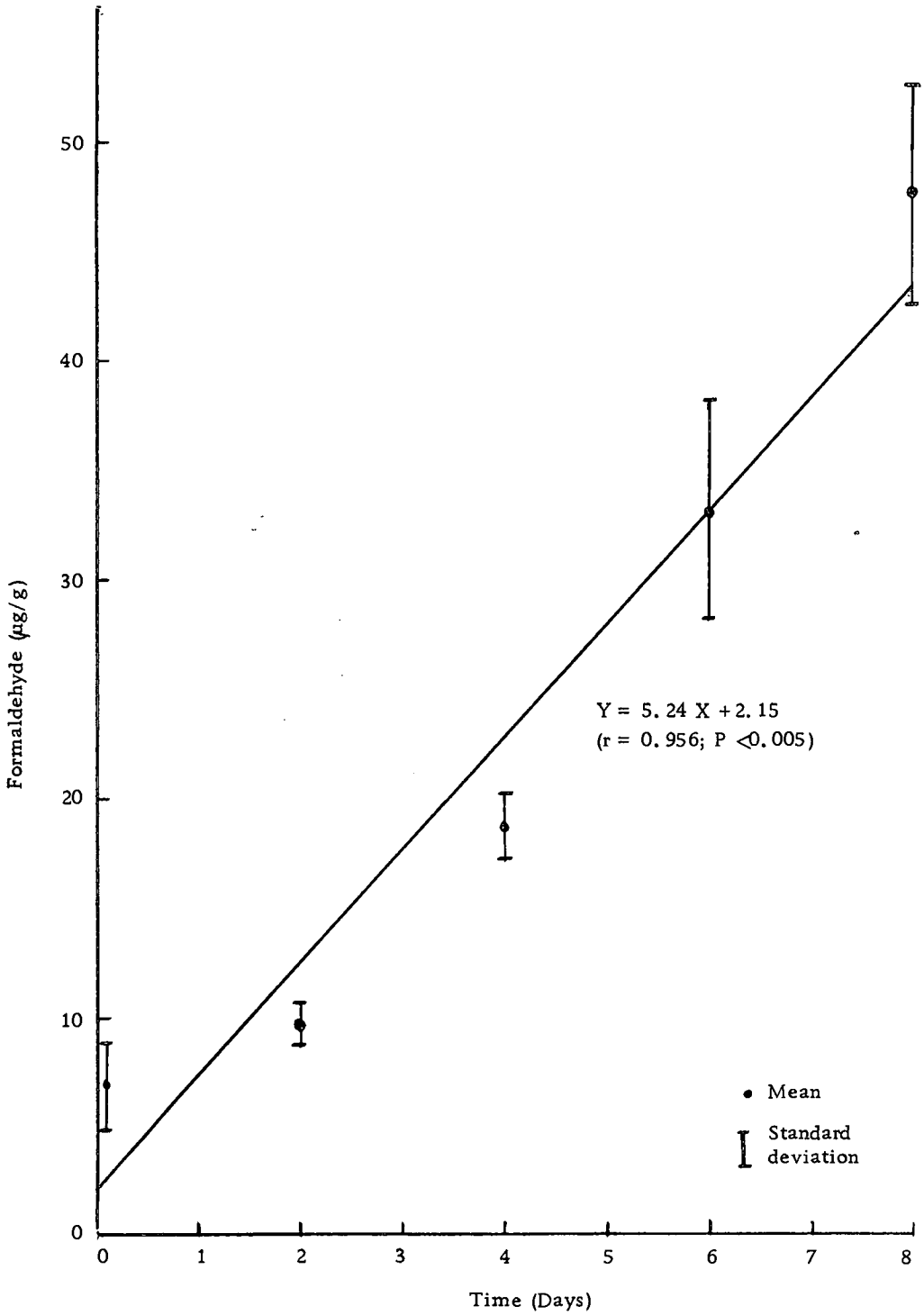

Figure 9. Regression of formaldehyde of raw meat derived from iced whole shrimp on storage time.

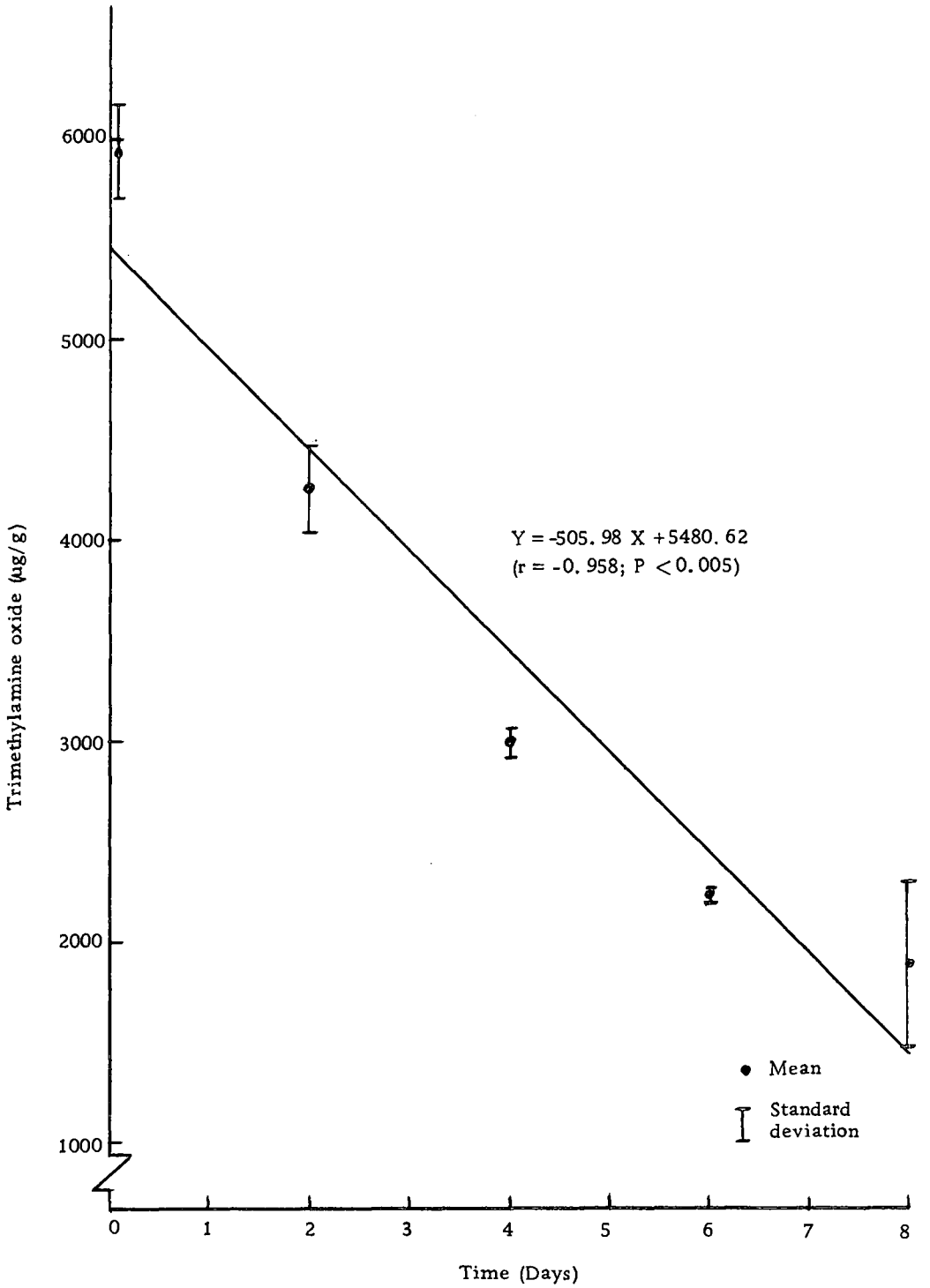

Figure 10. Regression of trimethylamine oxide content of cooked meat derived from iced whole shrimp on storage time.

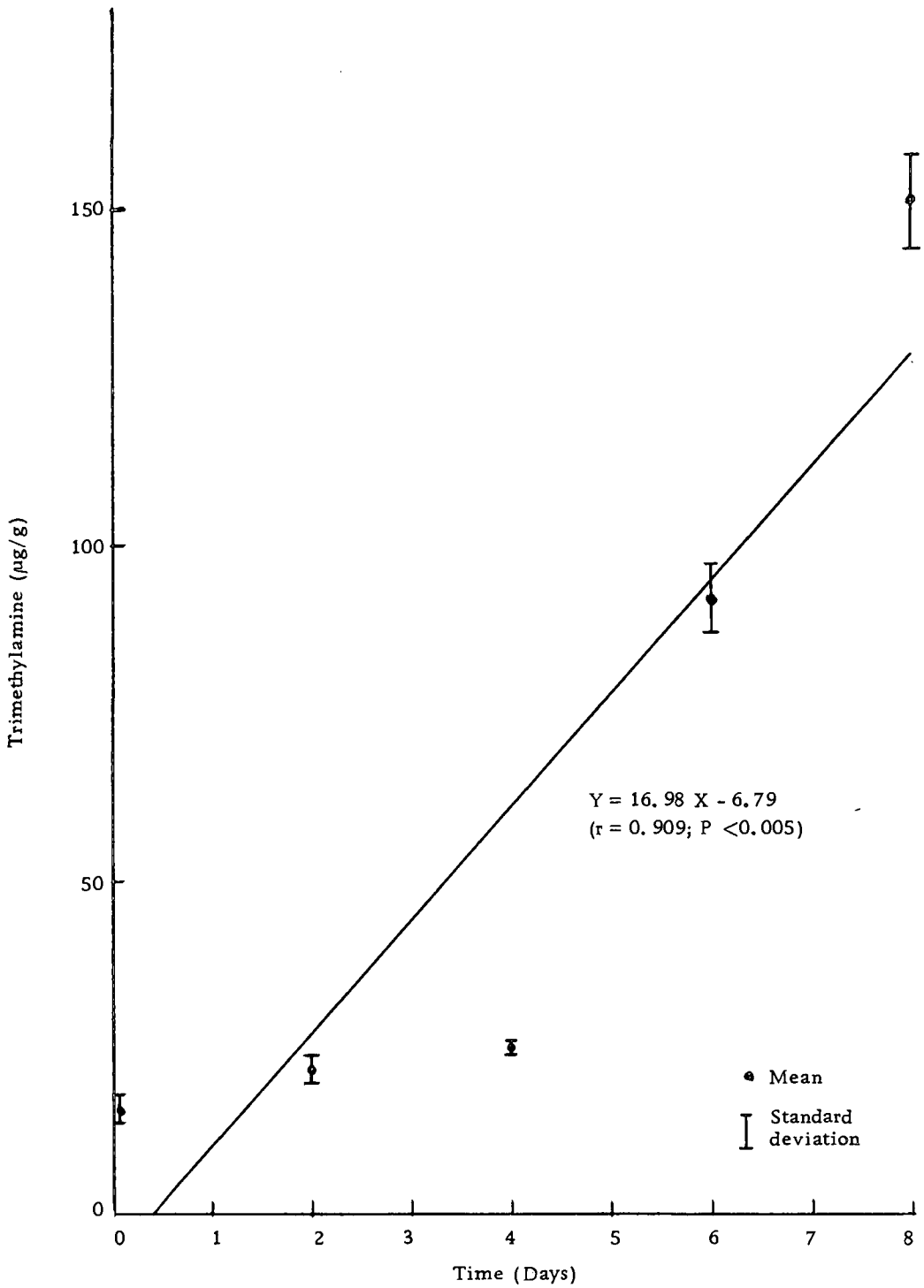

Figure 11. Regression of trimethylamine content of cooked meat derived from iced whole shrimp on storage time.

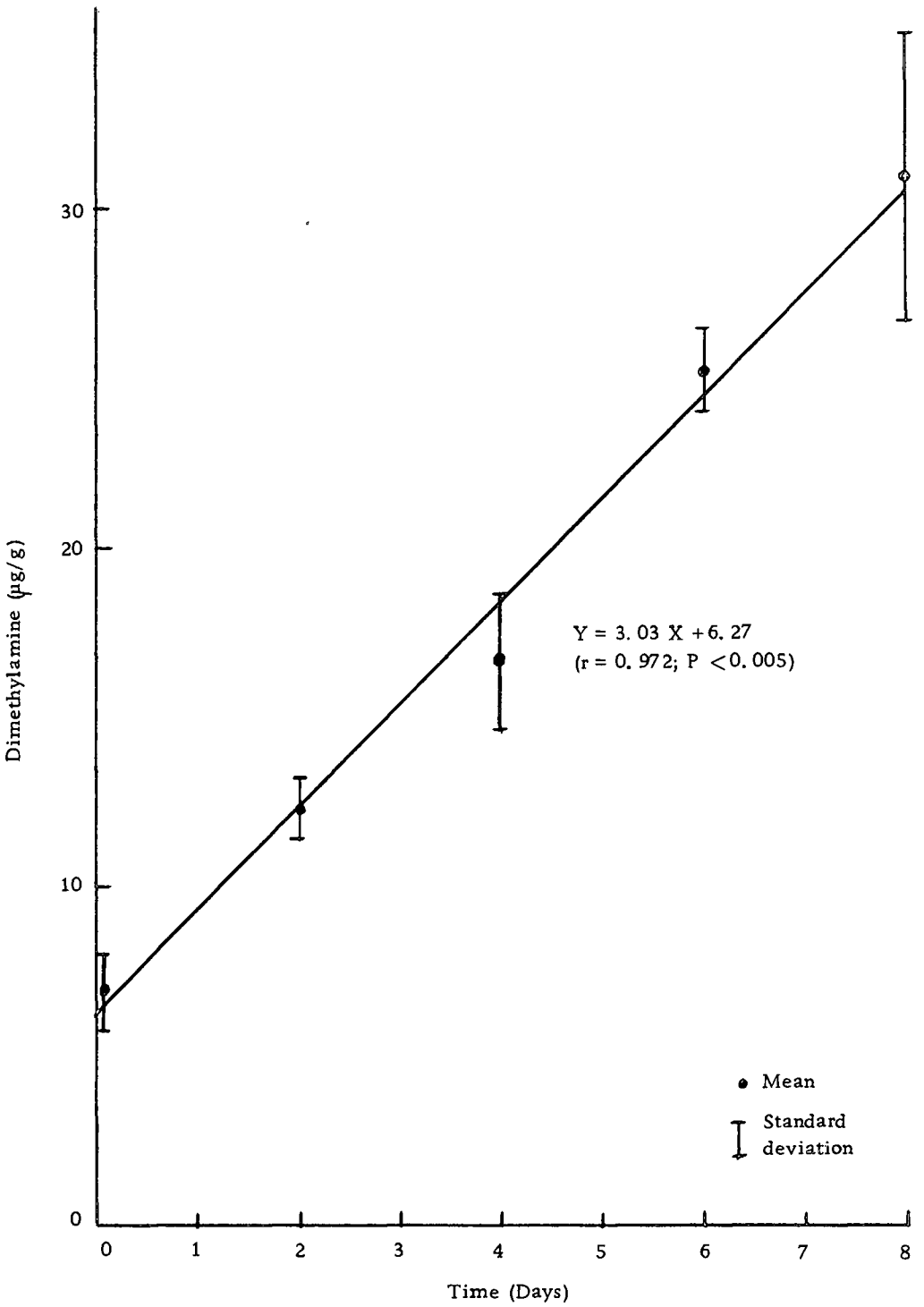

Figure 12. Regression of dimethylamine content of cooked meat derived from iced whole shrimp on storage time.

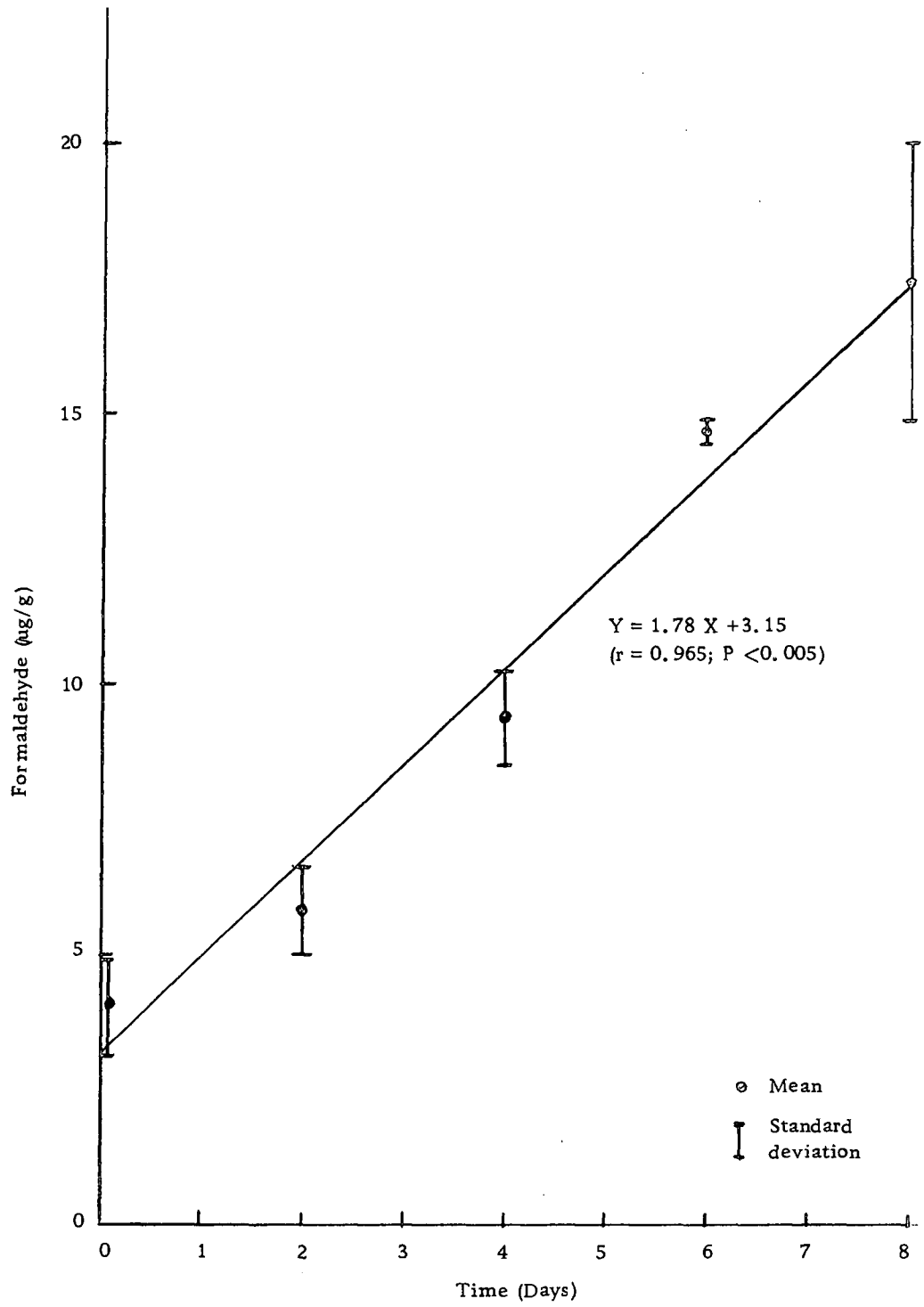

Figure 13. Regression of formaldehyde content of cooked meat derived from iced whole shrimp on storage time.

throughout the storage period. This indicates that most of the trimethylamine oxide is confined in the muscle and during cooking large losses of this compound occur. In general, the loss of trimethylamine oxide from the muscle during cooking, increased with the storage time. At the end of the storage period an apparent loss of 54% was observed over 39% at zero time.

Trimethylamine levels in the whole shrimp increased from 33 at zero time to 549 μg/g at the end of eight days of iced storage. Levels increased steadily during the first four days of storage, followed by rapid increase during the latter four days reflecting a rapid microbial out-growth. These results are not in agreement with the findings of Fieger and Friloux (1954), Bailey et al. (1956) for shrimp of the Gulf of Mexico and the observations of Iyengar et al. (1960) for shrimp from Indian waters. These investigations reported that a period existed during which the trimethylamine levels for iced shrimp did not show a definite trend and remained relatively constant. These differences in the production of trimethylamine in shrimp can be explained by differences in the handling. Shrimp from the Gulf of Mexico are headed after being caught removing the digestive organs. This operation has been reported by Green (1949) to reduce considerably the bacterial load of shrimp and increase its iced storage life by approximately ten days. As a result of this operation, a period of low bacterial load exists during which trimethylamine is

not produced. Pacific shrimp is stored in ice with heads on which make its initial bacterial load higher than shrimp from the Gulf of Mexico leading to a more rapid initial production of trimethylamine.

Qualitative and quantitative studies of the microbial flora of shrimp from the Gulf of Mexico reported by Green (1949) showed the flora at the beginning to be a mixture of genera, while at the end Achromobacter and Pseudomonas predominated. Haycock and Rieger (1971) have shown that most of the Achromobacter and Pseudomonas isolated from fish were able to reduce trimethylamine oxide to trimethylamine.

Trimethylamine levels in the raw and cooked meat were lower than those found in the raw shrimp, but followed the same general pattern of development. The reduction of trimethylamine oxide to trimethylamine by bacteria is essentially a surface phenomenon and only when spoilage is well advanced does the decomposition of trimethylamine oxide in the interior of the muscle occur (Watson, 1939). The difference in trimethylamine levels between whole shrimp and the raw meat could result from the diffusion of this compound to the gut of the shrimp and by losses during the peeling and washing. Lower trimethylamine levels in the cooked meat reflected losses during cooking.

Levels of dimethylamine and formaldehyde in whole shrimp, and raw and cooked meat increased steadily throughout the storage

period and in general showed the same inter-relationships as those described for trimethylamine.

Results of this investigation reflected the general pattern findings reported by Flores and Crawford (1973), but dimethylamine levels were shown to be somewhat lower and trimethylamine substantially higher at the end of the storage period.

Decomposition of Trimethylamine Oxide in Refrigerated Shrimp Meat

Figures 14 through 17 show the regression of trimethylamine oxide, trimethylamine, dimethylamine and formaldehyde levels, respectively, on refrigerated (1-2° C) storage time of raw shrimp meat. Levels of trimethylamine oxide decreased and trimethylamine, dimethylamine and formaldehyde levels increased in a linear manner ($P < 0.005$) with storage time.

Trimethylamine oxide levels (Figure 14) decreased from a zero time level of 7557 to 4675 μg/g at the end of the storage period. During the first four days of storage, the level of trimethylamine oxide decreased at a relatively slow rate. After this period, a sharp decline in trimethylamine oxide level occurred.

Trimethylamine levels (Figure 15), during the first four days remained relatively constant and corresponded to the same period during which trimethylamine oxide levels decreased at a relatively

slow rate. Trimethylamine levels then increased rapidly during the same period that a sharp decline in trimethylamine oxide levels occurred. Fieger and Friloux (1954) and Iyengar et al. (1960) obtained similar results for the increase of trimethylamine on ice stored headless shell shrimp. They found that significant increases in bacterial counts preceded increases in trimethylamine levels.

Both dimethylamine and formaldehyde (Figures 16 and 17) levels increased rapidly during the storage period. The formation of formaldehyde was parallel to that of dimethylamine, but was not equimolar. Similar results were found by Flores and Crawford (1973) for whole shrimp, and by Amano and Yamada (1964) for three species of gadoid fish held at refrigerated temperatures. Amano and Tozawa (1969), and Babbitt et al. (1972) reported that formaldehyde cannot be determined quantitatively in fish tissue because it reacts irreversibly with proteins and other tissue components.

At the end of the storage period a decline of formaldehyde levels was observed. Amano and Yamada (1964) observed a similar decrease after spoilage of the flesh took place. They speculated that a bacterial dehydrogenation of formaldehyde may be responsible for this decrease.

The disappearance of trimethylamine oxide and the formation of its decomposition products did not correlate well. The apparent decomposition of trimethylamine oxide was greater than the formation

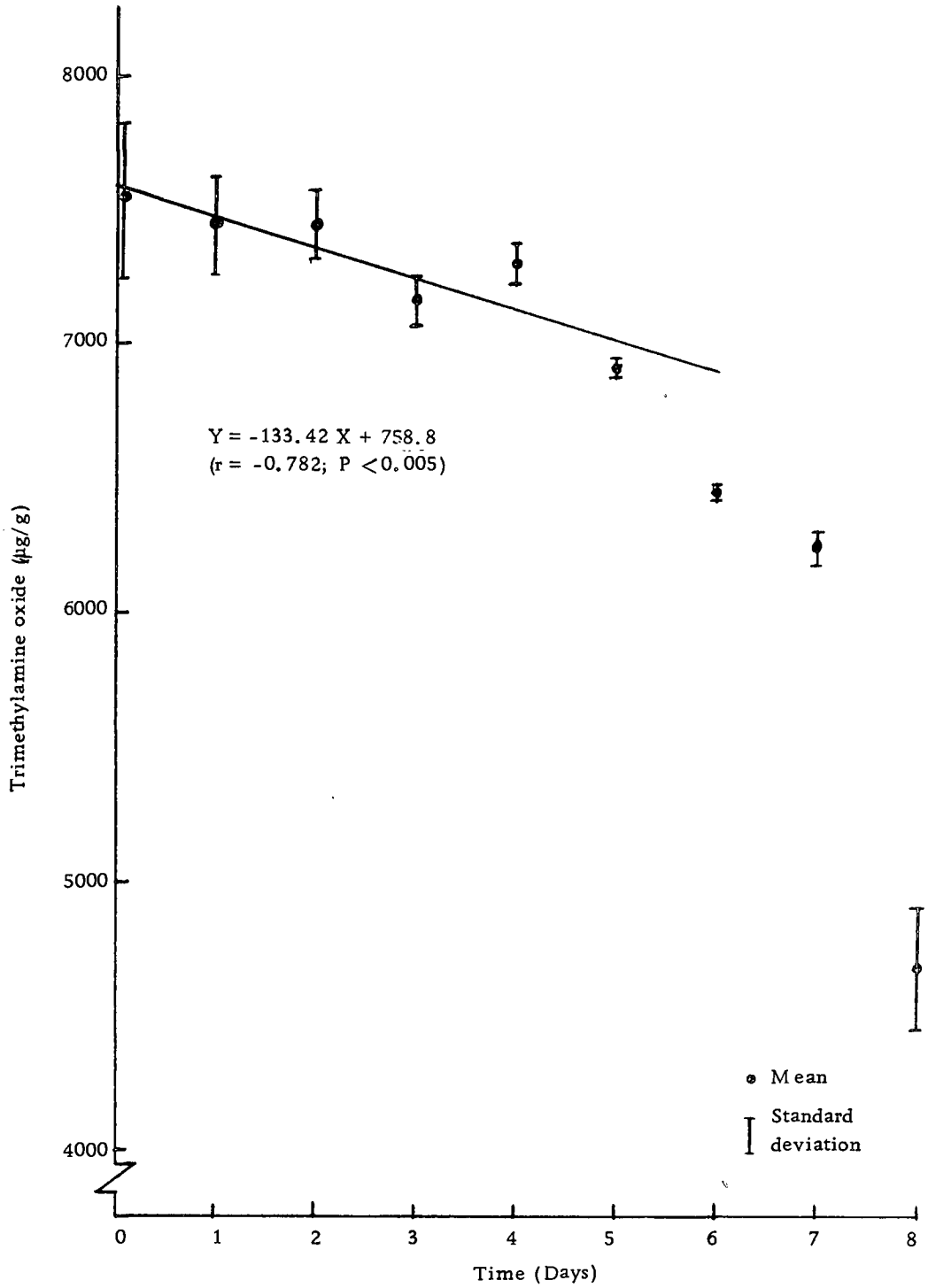

Figure 14. Regression of trimethylamine oxide content of raw shrimp meat on storage time at 1-2° C.

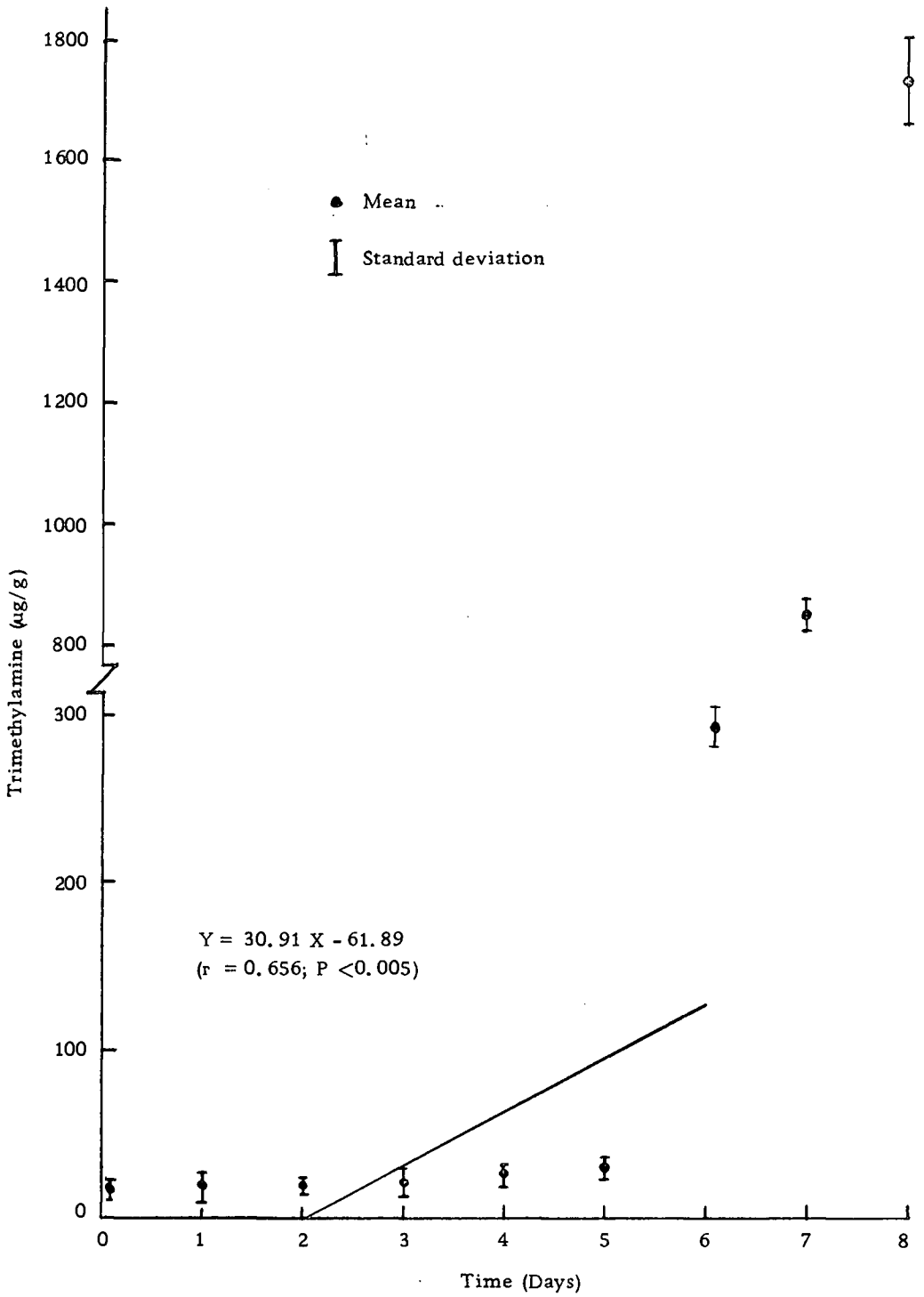

Figure 15. Regression of trimethylamine content of raw shrimp meat on storage time at 1-2° C.

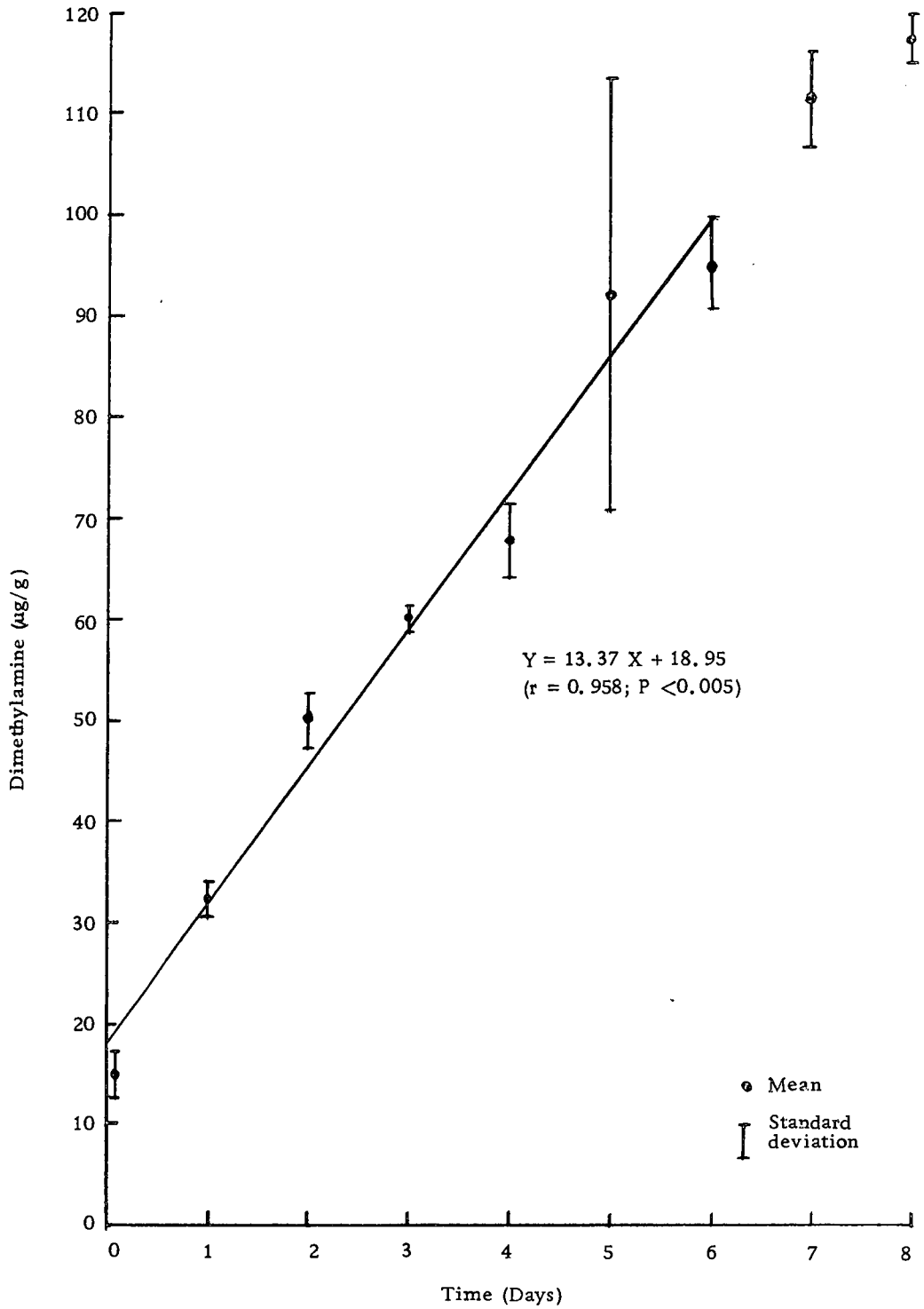

Figure 16. Regression of dimethylamine content of raw shrimp meat on storage time at 1-2° C.

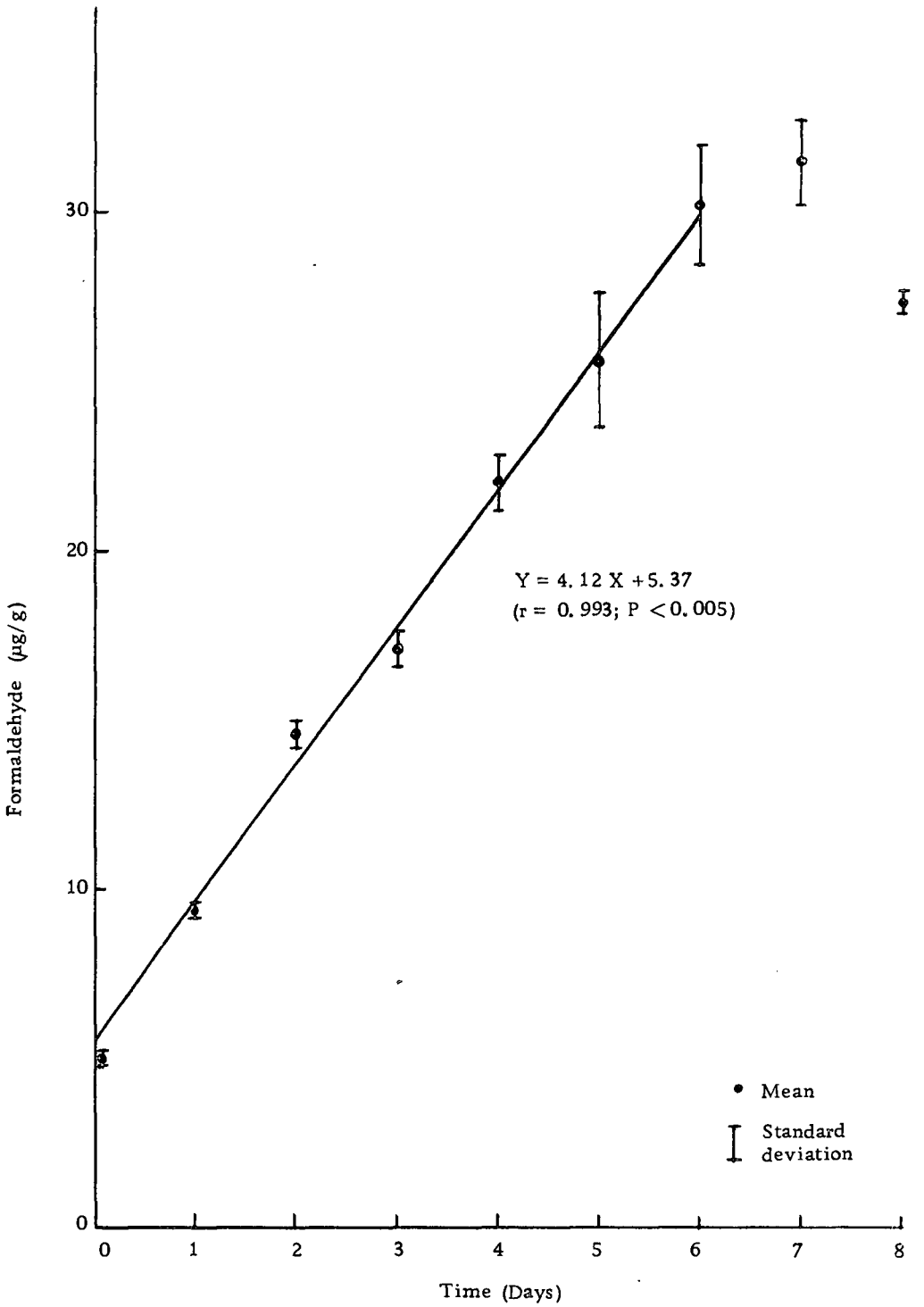

Figure 17. Regression of formaldehyde content of raw shrimp meat on storage time at 1-2° C.

of trimethylamine and dimethylamine combined. This disagreement can be accounted for by the variability of trimethylamine oxide levels in the samples and the loss of amines into the drip observed in the polyethylene bags during the storage. Hilling et al. (1963) reported that the drip from frozen fish usually yielded trimethylamine levels comparable to those for fillets.

The linear nature of the increase of dimethylamine and formaldehyde from the beginning of the storage period and the relatively constant levels of trimethylamine during the early period of storage lends support to the hypothesis that an enzymatic system similar to that found in gadoid fish (Amano and Yamada, 1964; Castell et al., 1970; Babbitt et al., 1972; Smith et al., 1975) which is responsible for the formation of dimethylamine and formaldehyde is present in Pacific shrimp.

Decomposition of Trimethylamine Oxide in Frozen Raw and Cooked Shrimp Meat

The rate of trimethylamine oxide decomposition in raw shrimp meat at temperatures below freezing is dependent upon the initial concentration of trimethylamine oxide.

Raw shrimp meat with high, medium and low initial trimethylamine oxide levels derived from whole shrimp stored on ice for zero, four and eight days (Table 2) yielded a linear ($r=0.981$,

$P < 0.005$; $r = 0.949$, $P < 0.005$ and $r = 0.619$, $P < 0.01$; respectively) formation of dimethylamine (Figures 18, 19 and 20, respectively) with apparent rates (Table 2) that correlated significantly ($r=0.992$; $P < 0.005$) with initial trimethylamine oxide levels.

The rate of dimethylamine formation in raw shrimp meat at -18° C was relatively slow. At 1-2° C shrimp meat with comparable initial trimethylamine oxide levels yielded a rate approximately 23 times (Figure 16) that observed at -18° C (Figure 18).

Formaldehyde levels in raw shrimp increased during storage (-18° C), but yielded a linear relationship only at high and medium initial levels of trimethylamine oxide ($r=0.713$, $P < 0.005$ and $r=0.561$, $P < 0.025$; respectively). The formation of formaldehyde with respect to time at low initial trimethylamine oxide levels did not provide a linear relationship ($r=0.216$, NS $P < 0.05$). The apparent rate of formaldehyde formation did not appear to be related to initial trimethylamine oxide levels. This result may have been due to a combination of the loss in homogeneity of the whole shrimp sample during ice storage and irreversible reaction of formaldehyde with muscle proteins. The loss of sample homogeneity during the iced storage of whole shrimp is clearly demonstrated by the increase in the standard deviations for dimethylamine mean levels observed for frozen raw meat samples frozen at high, medium and low initial trimethylamine oxide levels (Figures 18, 19 and 20, respectively).

A similar, but more variable pattern was found for formaldehyde levels.

Table 2. Dependence of the rate of DMA[1] and FA[2] formation on the initial concentration of TMAO[3] in raw shrimp meat at -18°C.

Whole shrimp			
Storage time (days)[4]	0	4	8
TMAO[5]	5169±92.0	3590±112.3	1847±176.9
TMA[5]	36± 1.8	65± 2.6	191± 24.8
DMA[5]	24± 3.9	59± 1.5	90± 9.2
FA[5]	9± 0.5	20± 1.7	37± 3.1
Raw frozen meat[6]			
Initial TMAO level (μM/gm)	111.8±20.6	58.2±8.4	20.6±1.9
DMA formation (μM/g/day)x10^3	12.285	8.293	2.272
FA formation (μM/g/day)x10^3	1.433	1.529	-

[1] Dimethylamine

[2] Formaldehyde

[3] Trimethylamine oxide

[4] Iced storage

[5] μg/g

[6] Derived from whole shrimp at corresponding days of iced storage

The formation of trimethylamine could not be correlated with time. This result could reflect no formation or the very large sample variation observed.

The concentration of trimethylamine oxide in the frozen meat samples varied greatly. The change in its concentration could not

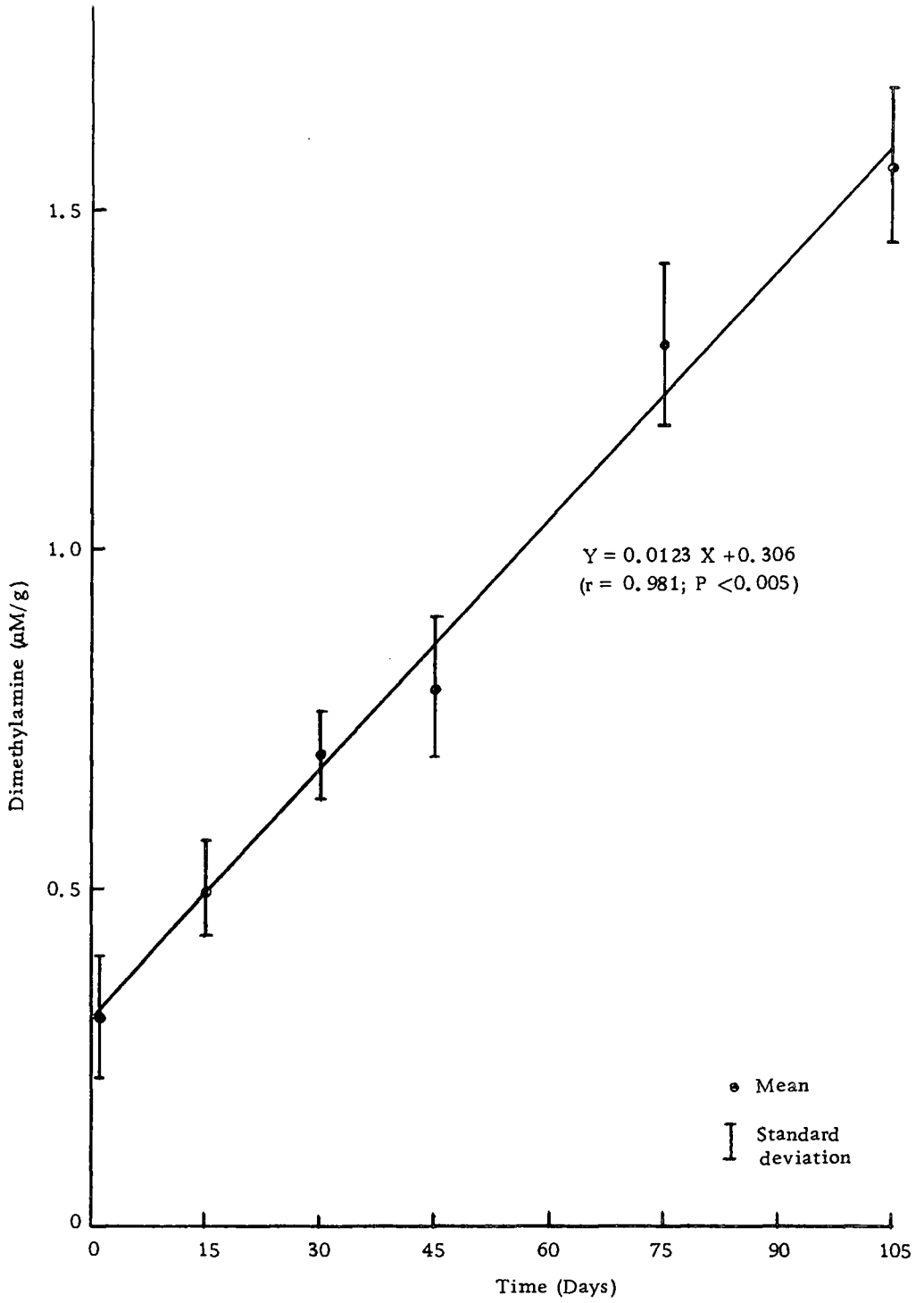

Figure 18. Regression of dimethylamine content of raw shrimp meat on storage time at -18° C. Initial trimethylamine oxide content of 111.8±20.6 μM/g.

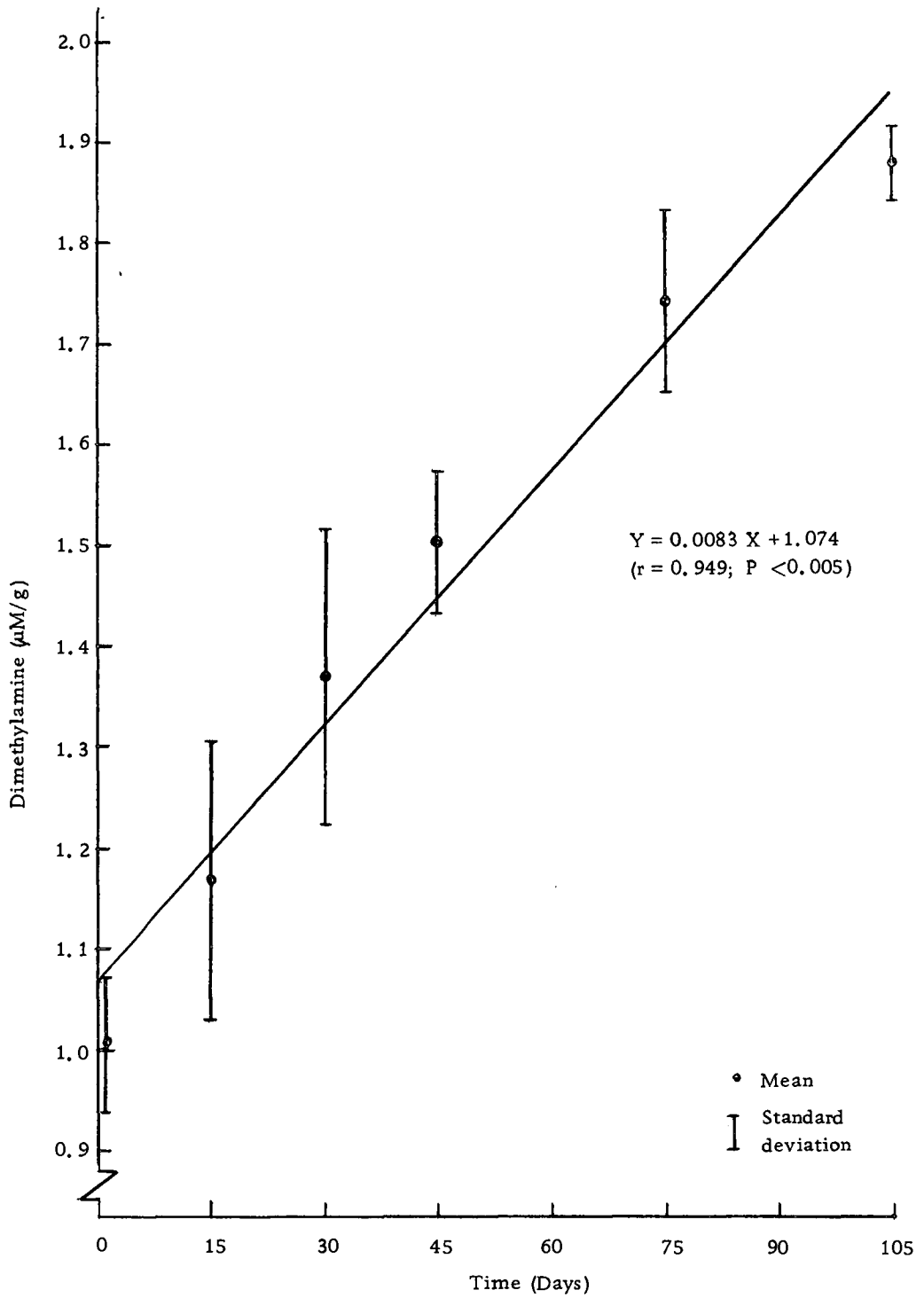

Figure 19. Regression of dimethylamine content of raw shrimp meat on storage time at -18° C. Initial trimethylamine oxide content of 58.2±8.4 μM/g.

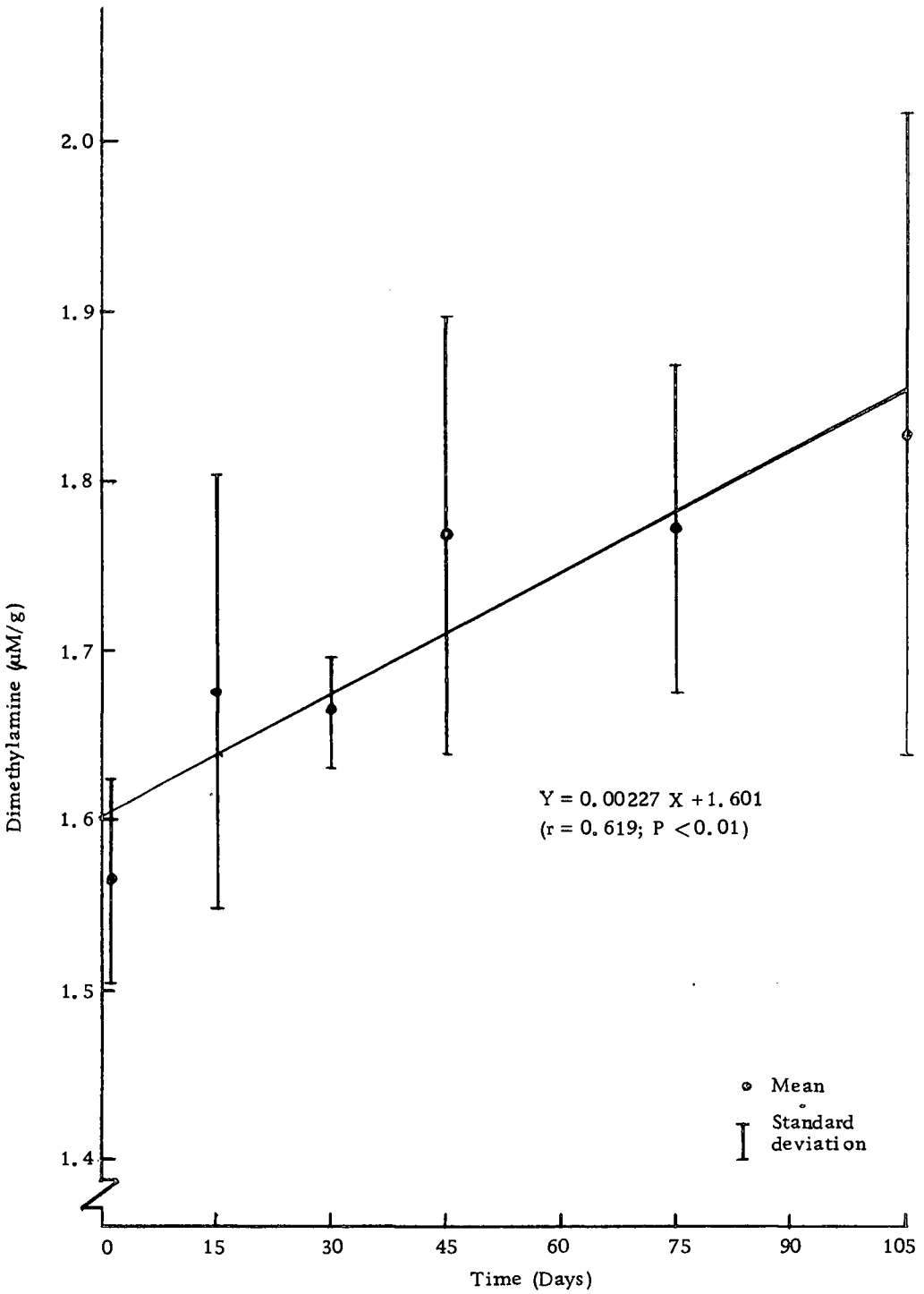

Figure 20. Regression of dimethylamine content of raw shrimp meat on storage time at -18° C. Initial trimethylamine oxide content of 20.6 ± 1.9 μM/g.

be correlated with time. Its high and variable concentration coupled with the relatively small amount of decomposition necessary to yield the products observed made its decomposition rate difficult to estimate.

The rate of dimethylamine formation in frozen raw shrimp meat appears to be dependent upon the initial concentration of trimethylamine oxide. This rate relationship, as the level of trimethylamine oxide, may only be a reflection of the storage time of the whole shrimp on ice. The concentration of the presumed enzyme system responsible for the formation of dimethylamine may have been greatly reduced by the washing action of melting ice. Considering the concentration relationships between trimethylamine oxide and its products of decomposition, the results obtained probably reflect an enzyme concentration relationship.

The mechanism responsible for the decomposition of trimethylamine oxide to dimethylamine and formaldehyde appears to be very heat sensitive. Raw shrimp meat cooked for 15 or 30 seconds at 100° C and held at -18° C yielded no apparent decomposition of trimethylamine oxide or production of dimethylamine or formaldehyde (Tables 3 and 4).

The presumed enzyme-catalyzed mechanism of dimethylamine formation in gadoid fish may be quite heat resistant. Yamada and Amano (1965) working with extracts from pyloric caeca of Alaskan

Table 3. Mean of TMAO,[1] TMA,[2] DMA[3] and FA[4] levels during frozen (-18° C) storage of shrimp meat cooked for 15 seconds at 100° C.

Storage time (days)	TMAO	TMA	DMA	FA
0	3972.46±455.39	10.08±2.55	13.23±3.14	6.33±1.41
15	3865.00±659.59	14.14±3.46	16.49±0.80	6.64±2.49
30	4632.67±340.58	13.62±0.61	12.98±2.52	6.74±0.99
45	4918.53±428.43	14.55±2.27	13.57±1.74	5.16±0.27

[1] Trimethylamine oxide

[2] Trimethylamine

[3] Dimethylamine

[4] Formaldehyde

Table 4. Mean of TMAO,[1] TMA,[2] DMA[3] and FA[4] levels during frozen (-18° C) storage of shrimp meat cooked for 30 seconds at 100° C.

Storage time (days)	TMAO	TMA	DMA	FA
0	4162.00± 271.73	12.95±3.17	13.86±2.35	4.55±0.81
15	4960.24± 525.57	17.35±0.95	20.70±2.20	4.75±0.20
30	4987.18±1116.22	13.88±0.17	13.90±2.25	3.75±0.22
45	4668.00± 266.51	15.36±1.95	13.02±2.57	5.59±1.07

[1] Trimethylamine oxide

[2] Trimethylamine

[3] Dimethylamine

[4] Formaldehyde

pollock, an active source of the dimethylamine forming enzyme, found that heating for five minutes at 100° C completely inhibited dimethylamine forming activity. Whether a shorter thermal treatment could inhibit the dimethylamine forming activity was not determined. Castell et al. (1971) reported that steaming for five minutes followed by autoclaving ten minutes at 110° C of gadoid fish fillets arrested considerably but did not stop the dimethylamine forming activity during subsequent storage at -5° C. Recently Smith et al. (1975) reported that pre-heating samples of silver hake to 80° C severely reduced the dimethylamine formation during subsequent storage at -10° C.

If the enzymatic system responsible for the formation of dimethylamine is not present in the muscle of shrimp but is confined to the intestinal tract, the shrimp meat may have been superficially contaminated with this enzyme during peeling. This would easily explain inhibition by these short thermal treatments. On the other hand, the combination of lower storage temperature used in this investigation and group variations in the enzyme properties could be the reason that dimethylamine was not formed in the heat treated meat.

Flavor, Panel Evaluations of Cooked Shrimp Meat and their Correlation with Levels of Trimethylamine Oxide and its Decomposition Products

Storage of whole Pacific shrimp on ice led to a steady decrease in the organoleptic quality of separated cooked meat. The regression of triplicate flavor panel mean scores for texture, juiciness, flavor and desirability for cooked meat decreased in a linear manner ($r=0.952$, $P<0.005$; $r=0.830$, $P<0.005$; $r=0.939$, $P<0.005$; $r=0.954$, $P<0.05$; respectively) (Table 5).

The juiciness and texture of cooked meat was least affected by iced storage. Scores for juiciness and texture were not significantly altered after two days iced storage. Scores for texture were significantly reduced from zero time values at four days, with a further significant reduction occurring on the eighth day. Juiciness scores were also significantly reduced on the fourth day of iced storage, bur remained unaltered from that time to the eighth day of storage.

The flavor and desirability of cooked meat was more uniformly affected by the iced storage of whole shrimp. Scores for flavor and desirability were significantly reduced for each two day increment of iced storage time.

The decomposition of trimethylamine oxide and the formation of trimethylamine, dimethylamine and formaldehyde in iced whole shrimp and in derived raw and cooked meat paralleled a

corresponding change in organoleptic acceptance. The correlation of the mean levels of trimethylamine oxide, trimethylamine, dimethylamine and formaldehyde in iced whole shrimp and raw and cooked meat derived by hand peeling (Table 6) with corresponding triplicate flavor panel mean scores for which average values are listed in Table 5 yielded the correlation coefficients listed in Table 7.

Table 5. Mean[1] flavor panel scores for cooked meat from whole Pacific shrimp stored on ice.

Storage time (days)	Scores[2, 3]			
	Texture	Juiciness	Flavor	Desirability
0	7.16±0.06[a]	7.03±0.16[a]	7.11±0.28[a]	7.15±0.17[a]
2	6.63±0.03[a]	6.57±0.18[a, b]	6.51±0.25[b]	6.44±0.15[b]
4	5.90±0.25[b]	6.35±0.27[b, c]	5.70±0.41[c]	5.72±0.40[c]
6	5.57±0.18[b, c]	5.92±0.28[c]	5.05±0.31[d]	5.09±0.28[d]
8	5.31±0.36[c]	6.03±0.35[c]	4.40±0.73[e]	4.55±0.58[e]

[1]Mean of average scores for triplicate flavor panels (n=15).

[2]Range of scores: 9, "extremely desirable," to 1, "extremely undesirable."

[3]Mean scores in a column with same exponent letter did not vary significantly ($P < 0.05$) from each other (n=45).

Table 6. Mean levels of TMAO,[1] TMA,[2] DMA[3] and FA[4] in whole shrimp and in raw and cooked meat from whole shrimp stored on ice.

	Storage time (days)	µg/g			
		TMAO	TMA	DMA	FA
Whole shrimp[5]	0	5141±342.6	33± 2.9	14±2.5	9±1.4
	2	4312±177.0	53± 0.7	31±3.1	20±3.9
	4	3353±251.0	64± 9.3	41±2.9	23±1.0
	6	2797±242.5	133±21.4	48±5.0	35±4.9
	8	2089± 89.7	549±38.0	81±2.2	49±3.0
Raw meat[6]	0	9783±361.5	25± 2.7	9±1.1	7±2.0
	2	7748±453.5	28± 0.7	16±1.4	9±1.0
	4	6318± 62.9	53± 8.8	35±0.8	19±1.5
	6	4499±269.8	81± 7.0	55±4.3	33±5.1
	8	4095±213.7	354±39.0	72±1.8	47±5.1
Cooked meat[7]	0	5938±233.4	16±1.1	7±1.2	4±0.9
	2	4248±216.3	22±1.5	12±0.8	6±0.8
	4	2979± 63.6	25±1.2	17±2.1	9±0.9
	6	2229± 13.0	92±4.8	25±1.1	15±0.1
	8	1888±406.3	151±7.5	31±4.2	17±2.6

[1] Trimethylamine oxide

[2] Trimethylamine

[3] Dimethylamine

[4] Formaldehyde

[5] n = 3

[6] n = 3

[7] n = 6

Table 7. Correlation coefficients for the regression of triplicate flavor panel mean scores for cooked meat on mean levels of TMAO,[1] TMA,[2] DMA[3] and FA[4] in whole shrimp and raw and cooked meat from whole Pacific shrimp stored on ice.

	Texture	Juiciness	Flavor	Desirability
Whole shrimp				
TMAO	.963*	.837*	.786*	.954*
TMA	-.677**	-.508	-.751*	-.740*
DMA	-.833*	-.729*	-.898*	-.905*
FA	-.903*	-.787*	-.915*	-.928*
Raw meat				
TMAO	.959*	.871*	.924*	.946*
TMA	-.680**	-.501	-.751*	-.739*
DMA	-.929*	-.798*	-.932*	-.939*
FA	-.892*	-.758*	-.913*	-.916*
Cooked meat				
TMAO	.961*	.866*	.909*	.935*
TMA	-.797*	-.681**	-.855*	-.848*
DMA	-.933*	-.823*	-.932*	-.945*
FA	-.929*	-.815*	-.929*	-.939*

[1] Trimethylamine oxide

[2] Trimethylamine

[3] Dimethylamine

[4] Formaldehyde

*Significant at $P < .005$

**Significant at $P < .01$

Trimethylamine oxide, dimethylamine and formaldehyde levels correlated very well with flavor panel scores ($P < 0.005$). Levels of trimethylamine did not correlate with same degree of significance or consistency. Amine and formaldehyde levels in the raw and cooked meat generally provided a superior correlation with flavor panel scores to those for whole shrimp. This was particularly true with regard to flavor. The significance of the correlation of amine and formaldehyde levels with flavor factors appeared to take the following general relationship: desirability>texture>flavor>juiciness.

Samples of cooked shrimp meat obtained from two different commercial processing plants and selected by subjective evaluation and age to represent the range of qualities currently processed by machine peeling yielded flavor panel scores that did not show a great deal of variation (Table 8). Their variation was significant, but did not reflect the wide differences shown for shrimp held on ice under laboratory conditions for eight days. Flavor panel scores and corresponding levels of trimethylamine oxide, trimethylamine, dimethylamine and formaldehyde in the whole shrimp and in the machine separated cooked meat (Table 9) indicated a range of qualities equivalent to 1 to 3-1/2 days iced storage post catch.

Correlation of the mean levels of trimethylamine oxide, trimethylamine, dimethylamine and formaldehyde in iced whole shrimp and mechanically derived cooked meat (Table 9) with corresponding

Table 8. Mean[1] flavor panel scores for cooked meat from whole Pacific shrimp processed commercially.

Replicate	Scores[2,3] Texture	Juiciness	Flavor	Desirability
1	6.71±0.69^{ab}	6.66±0.29^{a}	6.51±0.34^{ab}	6.42±0.32^{ab}
2	6.69±0.20^{a}	6.68±0.04^{a}	6.20±0.52^{a}	6.22±0.25^{a}
3	7.24±0.46^{c}	6.93±0.35^{ab}	6.93±0.12^{bc}	6.89±0.08^{bc}
4	7.31±0.14^{c}	7.11±0.33^{ab}	7.44±0.10^{c}	7.27±0.24^{c}
5	7.11±0.08^{bc}	7.33±0.17^{b}	7.11±0.20^{c}	7.04±0.20^{c}
6	7.40±0.18^{c}	7.29±0.36^{b}	7.20±0.31^{c}	7.20±0.29^{c}
7	7.42±0.20^{c}	7.15±0.56^{b}	7.24±0.28^{c}	7.22±0.38^{c}

[1]Mean of average scores for triplicate flavor panels (n=15).

[2]Range of scores: 9, "extremely desirable," to 1, "extremely undesirable."

[3]Mean scores in a column with same exponent letter did not vary significantly ($P < 0.05$) from each other (n = 45).

Table 9. Mean levels of TMAO,[1] TMA,[2] DMA[3] and FA[4] in whole shrimp and cooked meat from Pacific shrimp processed commercially.

	Replicate	μg/g TMAO	TMA	DMA	FA
Whole shrimp[5]	1	3702±871.2	52±5.9	16±2.3	10±0.6
	2	3377±198.6	48±3.4	22±3.8	18±1.5
	3	3873±174.1	44±4.9	13±1.8	11±1.0
	4	4382±227.8	46±2.2	10±2.9	7±3.0
	5	3894±64.6	57±9.0	19±5.6	14±5.2
	6	4841±258.1	46±3.0	10±1.5	8±1.5
	7	4423±232.8	35±2.7	13±1.0	9±1.4
Cooked meat[6]	1	3690±144.5	25±3.3	10±2.4	6±1.3
	2	3158±235.0	22±3.2	17±1.7	6±0.6
	3	3977±412.6	20±2.5	6±0.2	4±0.6
	4	4847±745.0	19±3.6	5±1.5	4±1.4
	5	4012±260.2	45±6.2	11±6.1	6±2.7
	6	5378±1022.7	21±2.9	5±2.2	3±1.0
	7	5223±400.8	17±1.3	6±1.9	3±1.1

[1] Trimethylamine oxide
[2] Trimethylamine
[3] Dimethylamine
[4] Formaldehyde
[5] n = 3
[6] n = 6

triplicate flavor panel mean scores for which average values are listed in Table 8 yielded the correlation coefficients listed in Table 10. Trimethylamine oxide levels correlated well ($P < 0.005$) with scores for desirability, flavor and texture, and was the only compound to correlate significantly ($P < 0.025$) with juiciness. Dimethylamine and formaldehyde levels also correlated significantly with texture, flavor and desirability, but the correlation coefficients were generally lower than those obtained for trimethylamine oxide. Trimethylamine did not correlate with the flavor factors.

The level of significance achieved for correlation derived from amine and formaldehyde levels in whole shrimp appeared to be nearly equal to that achieved by values for cooked meat. The significance of the correlation of amine and formaldehyde levels with flavor factors appeared to take the following general relationship: desirability > flavor > texture > juiciness. The significant correlations achieved with this rather narrow quality range in commercial samples indicates that even small differences between lots of shrimp can be detected.

Table 10. Correlation coefficients for the regression of triplicate flavor panel mean scores for cooked meat on mean levels of TMAO,[1] TMA,[2] DMA[3] and FA[4] in whole shrimp and cooked meat from whole Pacific shrimp processed commercially.

	Texture	Juiciness	Flavor	Desirability
Whole shrimp				
TMAO	.624*	.505***	.719*	.756*
TMA	-.405	.042	-.279	-.328
DMA	-.559**	-.295	-.677*	-.669*
FA	-.518***	-.287	-.658*	-.643*
Cooked meat				
TMAO	.643*	.498***	.738*	.776*
TMA	-.163	.215	-.032	-.043
DMA	-.618*	-.384	-.736*	-.740*
FA	-.660*	-.389	-.654*	-.704*

[1] Trimethylamine oxide
[2] Trimethylamine
[3] Dimethylamine
[4] Formaldehyde
*Significant at $P < .005$
**Significant at $P < .01$
***Significant at $P < .025$

SUMMARY AND CONCLUSIONS

Dimethylamine and formaldehyde are produced in a linear manner in whole shrimp and derived raw and cooked meat during iced storage of whole shrimp. The formation of dimethylamine and formaldehyde is accompanied by a simultaneous and linear disappearance of trimethylamine oxide. A similar linear system was found for separated raw meat at 1-2° C without ice. The formation of trimethylamine was less linear in nature and reflected more the logarithmic out-growth of bacteria.

The large difference between the initial and final levels of trimethylamine oxide and the lower concentrations of trimethylamine, dimethylamine and formaldehyde found at the end of the storage period in raw meat from whole shrimp stored on ice compared to those for separated raw shrimp meat stored at 1-2° C without ice, clearly demonstrated the extensive losses of amines during iced storage from the washing action of melting ice.

Formation of dimethylamine and formaldehyde in frozen raw meat (-18° C) was shown to be linear with respect to time. The rate of dimethylamine formation proved to be dependent upon the initial concentration of trimethylamine oxide in the tissue. Although a significant relationship with initial trimethylamine oxide was obtained, the rate dependency was more logically related to a decrease in

enzyme concentration accomplished by the washing action of melting ice on the whole shrimp from which the raw meat was derived. A similar concentration dependency could not be demonstrated for formaldehyde.

Heating raw shrimp meat in water at 100° C for 15 seconds completely inhibited the formation of dimethylamine and formaldehyde at -18° C. The presumed enzyme-catalyzed system for the decomposition of trimethylamine oxide appeared to be extremely heat sensitive.

Flavor panel scores for the quality of cooked meat derived from whole shrimp declined in a linear manner during iced storage. Scores for flavor and desirability showed the greatest magnitude of change.

Levels of trimethylamine oxide, dimethylamine and formaldehyde in whole shrimp and separated raw and cooked meat correlated well with flavor panel scores for cooked meat from shrimp stored on ice in the laboratory and from commercial processing operations. These indices proved to be more reliable indices of quality than trimethylamine.

Trimethylamine oxide is present in large amounts in shrimp tissue. Its disappearance through the action of tissue enzymes, microbial decomposition and the washing action of melting ice clearly reflects the storage conditions and quality of shrimp and yields a

large magnitude of change providing a precise chemical indice.

The use of trimethylamine oxide as a chemical indice meets required criteria for quality control work. It is accurate and precise; easy to perform and interpet; results can be obtained in a relatively short time; and does not require sophisticated instruments and techniques.

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